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Volume 20 NOVEMBER-DECEMBER, 1955 Number 6

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INSIDE SCIENCE

The Vital Story of VITAMIN A

by Science Writer

We live in a golden age of the sciences!

In the science of nutrition discoveries of food elements and their action have resulted in the promise of better health for us all.

Let's consider vitamin A as an example.

A Capsule History. 1913 was the year. Two teams working independently with animal feeding experiments discovered that a diet which was good in all other respects but which varied only in the source of fat caused young animals to thrive or sicken as the fat was varied. The work of these teams (McCollum and Davis; Osborne and Mendel) led to the discovery of the food factor which we know as vitamin A.

Composition and Action. A pale yellow, oil-soluble substance, vitamin A is expressed in the chemist's shorthand as $C_{20}H_{29}OH$. It is necessary in the diets of men and animals to promote the body's growth and development. Vitamin A is a vital requirement in guarding the health of the eyes and skin, and for resistance against infection. It helps maintain the health of mucous membranes and other specialized epithelial and glandular tissues.

Vitamin A enters the body as a constituent of food. Or it may be created within the body when a precursor of vitamin A is eaten. A precursor is a food element with potential vitamin A *value* but no actual vitamin A *content*. For instance, many green leaves have a high vitamin A value but, paradoxically, contain no actual vitamin A. The body converts the precursor into the vitamin. The most common precursor of vitamin A is beta carotene, the natural yellow coloring of many foods, such as dairy products, cereal grains, carrots, alfalfa, etc.

When consumed in food vitamin A is absorbed primarily in the healthy intestine, is esterified in the intestine's walls and is carried as an ester in the lymph and blood to the body's storage depots. The liver, which has a large capacity for storage, contains about 90% of the vitamin A found in the body.

Isolation and Synthesis. It was not until 1937 that pure, crystalline vitamin A was isolated by Holmes and Corbett from fish-liver oil. Research in the synthesis of vitamin A was stimulated during the Second World War by the threat to natural sources in fish-liver oil. This work resulted in several processes to produce vitamin A synthetically. Of these, one of the most successful was evolved by the Roche research team headed by Dr. Otto Isler which was announced in 1947. Much of the vitamin A now used for pharmaceutical manufacturing and food fortification in the United States and many other countries of the world is produced by this process.

Vitamin A and beta carotene produced by the Hoffmann-La Roche synthesis have many positive advantages for food and pharmaceutical manufacturers. Among these are the complete absence of any "fishy" odor, taste, or after-taste; the reasonable and stable price; their stability, purity and uniformity and the assurance of a steady economical supply.

Vitamin A is described in the U. S. Pharmacopoeia as a fully acceptable compound for use in pharmaceutical products. The U. S. Food and Drug Administration has approved its use for the fortification of margarine and other foods.

Human Requirements. The Food & Nutrition Board of the National Research Council recommends that a daily allowance of 5000 International Units of vitamin A in the diet will maintain good nutrition of healthy adults in the U.S.A. The Board's recommendations vary above this for pregnant and lactating women, and below for infants and children. The adult dose used in therapy may vary from 50,000 to 200,000 units daily.

Production. At the huge Roche Park plant of Hoffmann-La Roche in Nutley, New Jersey, over 500 pieces of large-scale chemical engineering equipment are working constantly to produce the superior 'Roche' vitamin A. This production is so large that 'Roche' vitamin A comes from the plant actually by the tons. In fact, the entire needs of the food and pharmaceutical industries of the United States and Canada can be met from this one plant.

Again the Roche people, who have so often led the way in vitamin research and production, have made another contribution to the better health and well-being of countless millions.

This article is one of a series giving a brief resume of our present knowledge of those essential health factors —the vitamins. Reprints of this and other articles are available without charge. The Vital Story of Vitamin C was published previously and others are forthcoming. Send your request to: Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul St., W., Montreal, Quebec.

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CHRONIC TOXICITY STUDIES ON ALKYL PHENYL PHTHALATE[a]

N. R. BREWER, A. J. CARLSON, AND ANNE PRATT
Department of Physiology, University of Chicago

(Manuscript received May 24, 1955)

A non-toxic chemical which exerts a significant effect as an emulsion stabilizer would be of great value in preventing losses affecting both the food industry and the consumer. Such a chemical would be particularly useful in preventing spoilage of a product of the nature of salad dressing, wherein crystallization and separation of the components is a problem. The use of alkyl phenyl phthalate (Alfenthal) to prevent this effect has been considered because of its efficiency as a crystallization inhibitor for stearins *(3)*. Although acute and subacute toxicity studies on rats, rabbits, and monkeys *(1)* have indicated that this compound is safe for use even at very high concentrations, the question as to whether or not repeated consumption continued over long periods of time would prove to have effects injurious to the organism remained to be determined. This report presents data collected in our laboratories in chronic studies over a period of 2 years in rats and 1 year in dogs, designed to test the possible chronic toxicity of alkyl phenyl phthalate (Alfenthal) in these animals.

METHODS

Rats. Sprague-Dawley rats, 80 weanling males and 80 weanling females (21 days of age) were divided into 4 groups, each group being made up of 20 males and 20 females, and housed 10 of one sex per cage. The individual rats were identified by a number tattooed on the tail. Blood determinations were made in duplicate for 6 male and 6 female rats from each group. When the rats were 28 days old, they were taken off regular rations and started on the experimental diet consisting of 61.1% whole ground wheat, 23.7% dried skim milk, 10% prime rendered lard, 4% cellulose plus Alfenthal mixture, 1% salt mixture (U.S.P. XIV), 0.1% Myva-Dry (20,000 units A/g.), 0.05% Delsterol (2,000 units D/g.), 0.002% alpha tocopherol, and 0.05% Wilson 1-20 liver powder (N.F. IX). The amounts of Alfenthal were incorporated into the diets by varying the proportions of cellullose to Alfenthal in the cellulose-Alfenthal mixture, and were as follows: Group I, 4% cellulose and 0% Alfenthal; Group II, 3% cellulose and 1% Alfenthal; Group III, 2% cellulose and 2% Alfenthal; Group IV, 0% cellulose and 4% Alfenthal. In addition to this, each group was fed 5 g. of fresh lean meat once per week and 5 g. of fresh lettuce leaf once per week. The rats were fed *ad libitum* and the food and water changed daily. The cages were sterilized weekly, and the rats were moved from one part of the room to another to randomize environmental factors such as light, heat, drafts, etc. After 3 months had elapsed, 10 females from each group were mated with 5 males from the same group, each male being placed in a cage with 2 females. These will hereafter be referred to as the P_1 generation. The offspring from these first matings were kept until the age of weaning, about 21 days, and then all surviving litters were destroyed. These litters will hereafter be referred to as the F_{1a} generation. Two months later the mothers were again mated to the same males. At the time of weaning 10 females and 5 males from each group of these second litters were saved and the rest of the litters sacrificed. These rats will be referred to

[a] This work was done under a grant from Swift and Company, and from the Douglas Smith Foundation for Medical Research of the University of Chicago.

as the F_{1b} generation. On reaching sexual maturity, the F_{1b} rats were mated and the resulting litters, hereafter referred to as the F_{2a} generation, were kept until weaning and then sacrificed. Two months later the F_{1b} rats were mated again and the offspring of this mating, the F_{2b} generation, were kept until they were weaned (approximately 21 days of age) at which time they were sacrificed and 6 males and 6 females from each group were necropsied. The F_{1b} generation rats were sacrificed when they reached the age of 6 months. The P_1 generation rats were maintained for a total period of about 2 years, at the end of which time they were sacrificed. All experimental animals were examined grossly by the pathologist at the time of sacrifice and histological sections of the following tissues were prepared and examined: heart, liver, lung, spleen, kidney, adrenal, pancreas, stomach, small intestine, thyroid, bone marrow, gonads, lymph nodes, and fat. Terminal blood studies consisting of red cell count, white cell count, differential, and hemoglobin determination were done on all rats at the time of sacrifice. Individual rats which died during the course of the experimental period were subjected to necropsy to determine the most probable cause of death.

Dogs. Twelve pure bred beagles from 3 litters were divided into 3 groups so that each litter was represented in each group. After 10 days on regular rations, the dogs in Group I were fed the following diet: 31.8% fresh ground horsemeat, 31.8% whole wheat bread, 21.2% water, 0.53% iodized salt, 2.12% steamed bone meal, 1.06% alfalfa leaf meal, 1.06% B-Y 500 vitamin B complex, 0.002% alpha tocopherol, 0.004% Myva-Dry (20,000 units A/g.), 0.04% Delsterol (2,000 units D/g.), 5.6% lard, and 4% ruffex. For Group II, Alfenthal was substituted in this diet to the extent of 2% by weight, replacing 2% ruffex, and the dogs in Group III received Alfenthal to the extent of 4% by weight of the total diet, replacing all the ruffex. In the case of Group III, it was noted that the dogs were not eating as well as the ones in the other groups. For this reason, the Alfenthal in the food for Group III was reduced to the level of Group II and the difference made up in gelatin capsules containing Alfenthal, which were placed in the food. With this method, the dogs ate their food more avidly and by watching them eat, the caretakers were able to be sure that the capsules were consumed. The dogs were fasted one day out of seven. The food was made up 3 times per week. At bi-monthly intervals, blood was drawn with a heparinized syringe from a vein in the foreleg and red cell, white cell, and differential counts and hemoglobin determinations were made. At the end of one year on the experimental diets, the dogs were sacrificed and terminal blood studies and necropsies were performed. Specimens were taken of heart, liver, lung, spleen, kidney, adrenal, pancreas, stomach, small intestine, thyroid, bone marrow, gonad, and lymph node, and were subjected to histological examination. In all cases code numbers were given the animals which bore no relation to their experimental treatment.

RESULTS

Rats. The final weights on the P_1 generation animals after 2 years on a diet containing up to 4% Alfenthal were not statistically different from the controls. Early in the experiment the gain in weight of those P_1 animals receiving the greatest amounts of Alfenthal in the diet was slightly less than those of the controls. The F_{1b} rats showed no statistically significant differences in weight among the 4 groups at any period in their growth. A compilation of weight averages for both generations is given in Tables 1_A and 1_B. The P_1 animals showed a high incidence of mammary tumors, bronchiectasis, and hemorrhage into the peritoneal or thoracic cavities or the small intestine in all groups. The F_{1b} generation did not show any of these conditions overtly, but bronchiectasis showed up with an equal frequency in all groups on necropsy. Mortality in the P_1 generation was high, but with no significant differences between the groups. Table 2 gives the mortality per group, per 6-month period. There were no deaths in the F_{1b} generation during the 6-month test period. The histopathologic findings were negative except for some focal pancreatic atrophy and arteritis appearing in the test groups but not in the control group of the P_1 animals. The only significant findings in the terminal blood studies occurred in the P_1 generation and consisted of a much lower eosinophil count in the test groups as follows: Group I showed an average of 1.4 eosinophils per 100 white cells counted, and Group II showed an average of 0.25 eosinophils per 100 white cells counted, while no eosinophils were noted in the surviving animals in Group III and Group IV. This pattern was not seen in the F_{1b} or the F_{2b}

TABLE 1A

Results of bi-monthly weighings, P_2 generation

(All values are average values for the noted sex and group and are in grams)

M—Mean S—Standard deviation

	Group I				Group II				Group III				Group IV			
	male mated celibate		female mated celibate		male mated celibate		female mated celibate		male mated celibate		female mated celibate		male mated celibate		female mated celibate	
June 4	51		49		49		48		53		52		49		53	
July 8																
M	247.8	250.2	173.2	178.5	245.6	247.7	177.7	179.9	240.7	239.2	179.4	174.7	231.7	230.6	171.2	166.2
S	16.8	11.7	10.7	10.1	8.6	14.6	7.3	8.3	10.5	23.4	10.1	4.8	6.3	18.6	28.6	13.1
Aug. 8																
M	326.4	314.5	215.3	215.3	344.8	329.6	219.4	222.9	318.8	332.4	223.6	218.8	320.0	316.1	207.6	213 1
S	12.2	20.4	13.4	12.9	18.6	21.5	8.5	14.8	12.4	19.8	19.9	9.1	22.7	20.0	12.9	12.5
Sept. 9																
M	378.4	371.2	243.3	235.2	386.0	371.8	248.0	252.2	356.8	367.7	244.8	236.1	362.0	359.4	224.7	236.4
S	21.3	32.5	63.9	67.4	120.4	71.0	14.4	8.8	20.8	60.8	17.2	17.2	29.2	22.1	16.9	3.7
Oct. 31																
M	453.0	421.9	255.5	272.0	453.0	438.5	285.0	284.5	436.0	449.7	278.0	263.5	412.5	397.1	258.5	260.5
S	18.6	32.8	20.3	18.4	120.4	55.9	14.4	23.4	37.3	35.9	22.5	16.7	36.6	28.8	18.7	21.3
Jan. 2																
M	508.0	484.6	302.0	290.5	522.0	506.4	315.0	302.0	550.0	494.6	320.6	290.5	466.6	496.6	288.3	287.0
S	24.9	33.3	83.1	93.5	34.9	45.1	60.9	64.8	32.4	42.3	21.9	24.1	18.7	31.5	27.9	22.0
Feb. 4																
M	526.0	505.0	329.5	321.0	525.0	518.5	309.4	308.0	522.5	528.0	327.7	310.0	476.6	490.0	295.0	306.0
S	19.5	29.6	34.4	31.08	29.2	67.3	21.1	31.2	46.4	43.8	24.5	18.3	18.1	38.1	9.7	31.3
March 6																
M	566.0	546.9	345.5	329.0	520.0	533.6	327.7	310.0	570.0	544.6	332.2	318.0	520.0	511.6	308.0	320.0
S	19.5	35.8	29.6	25.6	27.5	38.0	25.5	40.0	57.1	51.9	23.7	32.3	36.1	44.7	19.3	10.4
April 26																
M	580.0	548.4	335.5	343.0	562.0	553.0	343.3	350.0	555.0	570.7	351.1	335.0	500.0	534.5	317.0	340.0
S	24.5	35.2	50.7	30.5	37.7	54.2	35.4	33.9	50.7	48.4	25.9	33.7	28.3	44.3	30.9	125.7
June 18																
M	557.0	586.2	391.1	393.0	542.5	547.0	372.2	365.5	562.5	580.0	376.6	365.5	460.0	531.8	332.0	366.0
S	58.7	20.7	44.1	30.5	28.7	92.2	37.9	55.2	33.1	49.3	34.0	42.6	141.7	47.7	34.3	42.9

TABLE 1A (continued)

	Group I				Group II				Group III				Group IV			
	male mated	male celibate	female mated	female celibate	male mated	male celibate	female mated	female celibate	male mated	male celibate	female mated	female celibate	male mated	male celibate	female mated	female celibate
July 25																
M	524.0	548.3	355.5	365.0	550.0	556.6	383.3	366.2	537.5	560.0	376.6	381.1	495.0	521.0	357.0	397.0
S	119.5	50.6	61.0	33.4	42.4	82.9	40.0	59.6	20.6	63.7	41.3	46.1	7.0	67.2	54.8	38.9
Sept. 9																
M	562.5	514.5	397.1	371.0	502.5	555.5	380.0	366.2	521.5	526.6	402.0	387.7	550.0	546.6	346.0	390.0
S	22.3	85.1	28.2	30.1	149.3	55.4	37.6	76.4	22.2	36.1	54.2	49.6	0.0	45.3	66.1	60.4
Nov. 21																
M	640.0	569.0	418.5	385.0	460.0	591.4	356.7	402.8	527.5	506.2	376.6	368.7	.....	471.4	351.1	404.2
S	14.2	247.6	32.4	34.1	70.7	251.9	81.7	34.1	60.7	96.2	65.8	47.2	.....	85.1	46.3	50.4
Jan. 16																
M	620.0	476.0	355.5	345.0	400.0	548.3	386.6	390.0	570.0	478.3	350.0	358.5	.....	505.0	341.1	401.6
S	14.2	110.2	203.5	60.6	113.2	92.5	51.1	31.0	.....	62.1	53.5	48.4	.....	30.0	56.9	78.8
March 25																
M	540.0	.....	396.0	424.0	470.0	555.0	410.0	385.8	560.0	465.0	337.5	384.0	.....	490.0	380.0	373.3
S	.....	.....	32.9	61.9	.....	54.5	77.9	78.3	.....	91.9	90.7	96.1	.....	28.3	189.8	100.8
April 17																
M	660.0	.....	380.0	415.0	410.0	535.0	402.5	422.5	560.0	440.0	336.6	350.0	.....	510.0	340.0	410.0
S	.....	.....	35.4	19.7	.....	54.5	91.1	25.0	.....	113.2	93.9	18.3	.....	.....	111.2	99.0

TABLE 1B

Results of bi-monthly weighings, F_{1b} generation (all values are average values for the noted sex and group and are in grams)

M—Mean S—Standard deviation

		Group I		Group II		Group III		Group IV	
		male	female	male	female	male	female	male	female
Feb. 4	M	228.0	157.0	234.0	156.0	254.0	159.0	232.0	155.0
	S	24.9	17.7	46.7	17.6	19.5	24.7	21.7	12.6
Mar. 6	M	328.0	210.0	311.0	195.0	332.0	204.0	324.0	194.0
	S	33.2	13.3	46.1	13.7	20.5	19.9	23.6	15.8
Apr. 26	M	406.0	240.0	406.0	242.0	424.0	242.0	398.0	237.0
	S	41.6	37.7	39.7	22.1	20.7	20.4	31.9	18.8

TABLE 2

Mortality in the P_1 generation

M—Male
T—Tumor
F—Female

B—Bronchiectasis
H—Hemorrhage
GU—Gangrenous uterus

	Group I	Group II	Group III	Group IV
June 4, 1952— Dec. 4, 1952	2 M—H total—2	1 M—H 1 F—H total—2	0 total—0	3 M—H total—3
Dec. 4, 1952— June 4, 1953	1 F—T total—1	4 M—H 1 M—T total—5	3 M—H 1 F—H 1 F—T total—5	4 M—H total—4
June 4, 1953— Dec. 4, 1953	5 M—B 1 M—H 2 F—B total—8	3 M—B 2 M—H 5 F—B 1 F—T total—11	5 M—B 1 M—? 1 F—B 1 F—T total—8	5 M—B 1 M—H 1 M—T 2 F—H 2 F—T total—11
Dec. 4, 1953— May 23, 1954	7 M—H 4 M—B 3 F—T 3 F—H 2 F—B 1 F—GU total—20	2 M—H 1 M—B 1 M—T 2 F—B 1 F—H 1 F—GU 2 F—T total—10	3 M—B 6 M—H 3 F—B 4 F—H 1 F—? total—17	4 M—H 1 M—B 6 F—H 5 F—B 1 F—T total—17
No. dead/No. in group	31/40	28/40	30/40	36/40

generations. Animals in all groups have a healthy, well-nourished appearance except where bronchiectasis or tumors caused emaciation. There were undue losses of offspring due to difficulties with the heating system during critical periods in the quarters where the rats were housed. Weights of mated and celibate animals in the P_1 generation did not differ significantly in any group (Table 1_A). However, Table 3 summarizes the data obtained. From these data there was no evidence that Alfenthal had affected the fertility of the rats.

Dogs. There were no significant differences in growth among the 3 groups of animals. There were no deaths in any of the groups, and no overt symptoms of any pathology were noted. The gross and microscopic findings in these animals are those which are expected in animals of this age, and no findings were seen in the experimental animals which also did not occur in the control group. The bi-monthly blood studies showed a transitory anemia, occurring in 3 dogs of Group III, the group which received 4% Alfenthal in the diet. This anemia was first detected 4 months after the dogs were put on the experimental diet. There was a drop in the red cell count to about 80% of normal seen in the blood studies at the fourth month and a drop to only 70% of normal at the sixth month. This drop in the red cell counts was accompanied by a correspondingly low hemoglobin reading. However, by the eighth month the transitory anemia had disappeared and it did not recur. During this time there was no change noted in the appearance or behaviour of these dogs. The animals in

TABLE 3

Matings and offspring

Frac.—Fraction of females mated who conceived and delivered.

Num.—Average number of offspring in litter at weaning.

Mating	Group	Frac.	Num.
1st mating of P_1	I	9/10	3.8
	II	7/10	2.3
	III	7/10	3.5
	IV	7/10	4.0
2nd mating of P_1	I	6/10	8.6
	II	8/10	7.6
	III	7/10	9.0
	IV	7/10	5.2
1st mating of F_{1b}	I	8/10	5.2
	II	6/10	5.6
	III	8/10	4.7
	IV	8/10	2.5
2nd mating of F_{1b}	I	7/10	6.5
	II	7/10	9.5
	III	9/10	9.8
	IV	7/10	9.0

Group II, receiving 2% Alfenthal in the diet, showed a significantly low leucocyte and high neutrophil count in blood studies at the eighth month of the experiment. These findings did not show up again in either the next bi-monthly determinations or in the terminal blood studies.

DISCUSSION

Acute toxicity studies in rats, rabbits, and monkeys *(1)* indicate that Alfenthal is not toxic to these animals even when administered in very large doses. It was found impossible to obtain an LD_{50} value for rats since doses as high as 34.2 g./kg. administered by stomach tube failed to kill the animals. It was found in acute studies on monkeys that single doses of Alfenthal up to and including 48.58 g. (15 g./kg.) were not toxic. Also, young male rats fed a diet containing 2% of Alfenthal exhibited normal growth over a period of 8 weeks, and when this test was continued for 11 months, the survival rates of the experimental animals were in no case lower than those of the control animals. Other tests done in these laboratories indicated that Alfenthal did not in any way affect the reproductive capacities of rats receiving as much as 2% Alfenthal in the diet over a period of one year.

These results agree very well with those found in our studies in the case of growth and life span. At the beginning of the experiment the Group IV rats gained weight more slowly than did the other groups. However, within 4 months their weights reached levels comparable with those of the control group. Other than this, there was no impairment in growth noted in either rats or dogs.

The focal pancreatic atrophy and arteritis found in test groups of the P_1 rats were not seen in the F_{1b} generation which was 6 months of age at the time of sacrifice. In the opinion of the pathologist, these changes do not constitute significant lesions suggesting differences due to the substance being tested. Necropsies performed on these animals did not indicate any conditions which were not also seen in the control animals, and these conditions are not uncommon in the strain of rat used. Too, when the mortality figures are broken down into 6-month periods (Table 2) there was no single period or group of periods when the mortality in Group IV was significantly higher than in the other groups.

Rats subsisting for 2 years on diets containing 1%, 2% and 4% Alfenthal showed lower eosinophil counts in the terminal blood studies than did the control animals. This is a difficult finding to interpret inasmuch as the best known response to some pathological conditions as allergic states, i.e. asthma, anaphylactic shock, and infestation by animal parasites is an increase in the number of circulating eosinophils. It is known that eosinopenia occurs during the acute stage of pyogenic infection but it certainly would not seem that this is the reason here.

The group of dogs which had been fed a diet containing 2% Alfenthal showed a transitory drop in the leucocyte count and an increase in the neutrophil count after 8 months. This condition vanished as suddenly as it appeared and the absence of a similar condition in animals receiving 4% Alfenthal in the diet for a similar length of time combined with the fact that there was no pathology which could be correlated with it would seem to indicate that little if any significance in terms of the substance tested exists. The 3 dogs that received 4% Alfenthal in the diet showed a transitory anemia that appeared 4 months after the beginning of the experiment and lasted about 4 months, then disappeared. Here again, there was no demonstrable etiology or pathology which could be correlated with this condition and its significance is doubtful.

The quantities of Alfenthal administered in these experiments were enormous in comparison to the 0.02% that would be used to inhibit crystallization of salad oil, and which would be diluted to 0.01% in mayonnaise. If the average daily per capita consumption of salad oil is 8.3 gm. (2), and all of this were treated with 0.02% Alfenthal, then the average daily consumption of Alfenthal would be 1.66 mg. For a person weighing 60 kg. the average Alfenthal intake would be 0.0276 mg/kg. On the basis of an average food consumption per rat of 15 gm/day, the rats fed 4% Alfenthal for 2 years had an average daily intake of 2000 mg/kg, constituting a safety factor of more than 72,000 to 1.

SUMMARY

Chronic toxicity experiments with alkyl phenyl phthalate have been carried out in rats for two years and on dogs for one year at dose levels greatly exceeding those to which man would be exposed if this compound were used commercially. The rats consuming a dietary level of 4% Alfenthal, an equivalent of more than 72,000 times that which would be consumed daily by man if this chemical were to be used in salad oils, revealed

no impairment in growth or life span and no significant gross pathology as compared with the rats in the control group. The dogs fed Alfenthal for one year showed no significant changes in growth, in hematological findings, nor in gross and histopathology.

Acknowledgment

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VARIATION IN FLAVOR JUDGMENTS IN A GROUP SITUATION[a]

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The origin of many laboratory procedures is often obscured by their gradual and simultaneous development throughout a particular discipline. Food technology in general and consumer acceptance testing in particular are no exception to this phenomenon. Thus we find ourselves surrounded with numerous methodologies or protocols, few of which have been systematically refuted or substantiated.

The focal point of many activities in a food laboratory is the consumer or expert panel, whose task it is to evaluate the technologist's work. This panel may assume many forms in many locations; it may consist of two judges or two thousand; it may function in a department store, home, mess hall, laboratory or conference room; it may employ rating scales, difference or preference tests, or may involve the classification of particular qualities. In short, then, it is a complex field of endeavor whose outermost boundaries are often negotiated with little more than good common sense.

One procedural problem, which many of us assume has been answered to our particular satisfaction, concerns the physical environment in which panel judgments are made. In our own laboratory we use exclusively a multiple testing station which, in addition to standardizing certain test conditions, assures the experimenter of individual judgments and reports.

Other laboratories which we have visited, and which are frequently described in the literature, employ a discussion or round-table situation in which reports are orally given and decisions hammered out. Somewhere between these extremes lies the home test location, in which a round-table atmosphere may be approached; or the store test station, in which the multiple laboratory environment may be achieved.

It was the authors' feeling that our own adherence to a particular procedure was perhaps no more rational than our colleagues' adherence to a procedure in which we had little confidence. This present experiment, then, is an attempt to compare systematically the two ends of the acceptance-testing continuum, with a view toward determining their relative desirability as test methods. This comparison of the round-table and individual test situations was designed to test the hypothesis that the observers' judgments in a round-table situation would be influenced by the responses of the other panel members, whereas the individual panel method would present an independent and non-manipulated judgment.

PROCEDURE AND RESULTS

Part I

The first part of this study was a direct comparison between the individual and round-table panel methods, utilizing the same observer panels and the same test foods. Thus, all conditions were identical from one situation to the other, except for the type

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of panel. In each case the same group of 36 observers was used. This group of men and women was drawn from the larger population of trained psychometric observers at our laboratory. These observers, selected for their sensitivity to odor and flavor qualities and differences, have participated in a great variety of problems involving analysis and classification of foods. They are highly familiar with flavor testing methodology, particularly as it relates to difference and preference testing procedures. Thus, since this group cannot be described as a panel of flavor experts, we refer to them as trained, as opposed to naive, observers.

Three standard foods were chosen for comparative analysis by the two methods: tomato juice, baked beans, and crushed pineapple. For the tomato juice, each observer was served 2 coded samples and asked, "Which is saltier?" The 2 samples differed only in salt content. For the baked beans, a preference judgment between 2 samples of different brands was required; and for the pineapple, the observers were asked to give a relative sweetness judgment between 2 identical samples.

For the "individual" panel method, the illustrated multiple testing station was utilized. Coded samples were served to the observer, and after tasting back and forth between the two, he recorded his judgment on a test blank and was then served the next pair of samples. Thus, all judgments were individually arrived at and privately reported.

For the "round-table" method, the observers were scheduled for testings in groups of seven. Each group, supervised by a recorder-experimenter, sat around a table and was presented simultaneously with the first pair of coded samples. They were instructed to report their judgments orally to the recorder as soon as they had

TABLE 1

Distribution of observer judgments under individual and round-table panel conditions

Test materials	Panel condition							
	Individual				Round table			
Tomato Juice	No salt	Salt	No salt	Salt	No salt	Salt	No salt	Salt
Judgments "saltier"	8	21	7	25	10	26[1]	18[1]	16
Baked Beans	A	B	A	B	A	B	A	B
Preference judgments	11	21	9	23	15	21[1]	15[1]	18
Pineapple	X	X	X	X	X	X	X	X
Judgments "sweeter"	14	14	17	15	6	30[1]	13	20[1]

[1] Sample chosen by "leader."

reached a decision. As the judgments were given, they were recorded by the experimenter, and the group then proceeded to the next pair of samples.

It will be remembered that our prediction was that, in the round-table situation, the observer's judgments would be influenced by the responses of the other panel members. Actually, of course, under the round-table condition there is a great number and variety of possible influences which might combine to affect a given observer's response; but in the present experiment, for the sake of simplicity, it was decided to isolate only one of these factors. The variable selected was the response of the first observer in the round-table group; our hypothesis, then, was that in the round-table situation a statistically significant number of judgments might be changed in the direction of the first-responding observer's judgment. If this were found to be true, we could predict that the overall distorting effect of majority opinion would be even greater.

In order to standardize the response of the first observer in each panel, 6 panel members were trained in advance as "leaders." Before each round-table session they were instructed as to which responses they were to give; then one was assigned to each group of 6 observers, with instructions to announce his response first in each case. All 6 "leaders" were selected from the population of regular observers. Their responses were given in a natural manner so that none of the other panel members ever suspected that they were not ordinary observers.

The responses of the "leaders" were, of course, not included in the tabulation of the data.

A total of 4 panel sessions was conducted as described above, two using the individual panel method and two with the round-table method. The sessions were separated by intervals of at least one week, thus making for small differences in panel size due to absences on test days.

TABLE 2

Distribution of observer choices between identical samples under round-table condition

Test materials	Session 1		Session 2	
Grapefruit Juice				
Judgments "more sour"	15	13[1]	3	30[1]
Spaghetti				
Preference judgments	6	22[1]	13	12[1]
Cranberry Jelly				
Judgments "sweeter"	11	17[1]	4	21[1]

[1] Sample chosen by first observer.

RESULTS

The results of this phase are shown in Figure 1, which presents, for each test food, the number of observers choosing each sample under the various test conditions. The round-table and individual panel situations are represented by black and white bars respectively; the arrows indicate the sample chosen by the "leader." Thus we have a picture of the variation in group response, if any, caused by the judgments of the "leader."

An examination of the first section of the figure, which gives the results obtained on tomato juice, reveals that such an effect did occur. Under individual panel conditions, we find that the group as a whole was consistently able to detect the salt in sample "S"; in each case, approximately three-quarters of the panel members chose the correct sample as being saltier. In the first round-table situation with the "leader" naming the correct sample, no variation from these proportions occurred; apparently the leaders' reports had no effect. With the leader naming the incorrect sample, however, a definite shift in group response occurs, and more than half of the observers are led to name the *less* salty sample as saltier. Using a Chi-square test of contingency, the difference between the first and second round-table situations is found to be significant at the .05 level of confidence. Thus we see that, while the individual panel results remain consistent from one session to the next, the results of a round-table method can be substantially changed depending on the report of the first panel member.

The second part of Figure 1 shows the results obtained from a preference test on 2 brands of baked beans. In this case, a preference for Brand B was obtained in greater or less degree throughout all four experimental conditions, and the introduction of a "leader" had no significant effect in shifting the group judgment one way or the other.

The most striking evidence of the effect of the "leader's" report is found in the test on crushed pineapple, in which a relative sweetness judgment was required on 2 identical samples. Under individual panel conditions, the expected results were obtained; in each case, the observers' choices were evenly divided between the 2 samples.

Under round-table conditions, however, an entirely different result was obtained. In each case, there was a shift of response in the direction of the leader's report, and in one case the striking split of 30 to 6 was obtained on the 2 identical samples. Normally, of course, a difference of this size would be taken as solid evidence that a perceptible flavor difference existed between the 2 samples, illustrating the extent to which round-table data can mislead the experimenter.

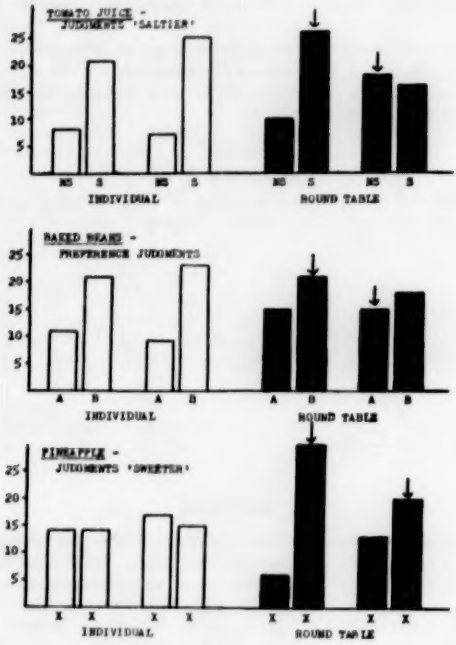

Figure 1. Distribution of observer judgments under individual and round-table panel conditions.

Part II

In view of the large differences obtained with pineapple, using identical samples, it was decided to conduct additional round-table comparisons of identical samples in order to demonstrate further the extent to which an observer group can be influenced.

PROCEDURE

A second set of round-table sessions was therefore run, using grapefruit juice, canned spaghetti and cranberry jelly as test foods. The observers were served 2 identical samples of each food and were asked to make judgments of relative sourness, preference, and sweetness, respectively.

Individual panel sessions were not conducted, since the results on pineapple in the previous phase had shown that 50-50 splits on identical samples could be expected. The round-table procedure was the same as before except that trained "leaders" were not employed. Instead, the experimenter noted the report of the observer who happened to respond first in each group, and then recorded the number of panel members whose report was the same or different. Thus the situation was an entirely natural one, since even the individuals who served as "leaders" were unaware that they were doing so. Their responses were not included in the final tabulations.

RESULTS

The results of the second phase are shown in Figure 2, the coding is the same as in the first figure. The first observer's report is indicated by the arrow in each pair. A glance at the 3 graphs shows that, in 3 out of 6 comparisons, the leader's judgment

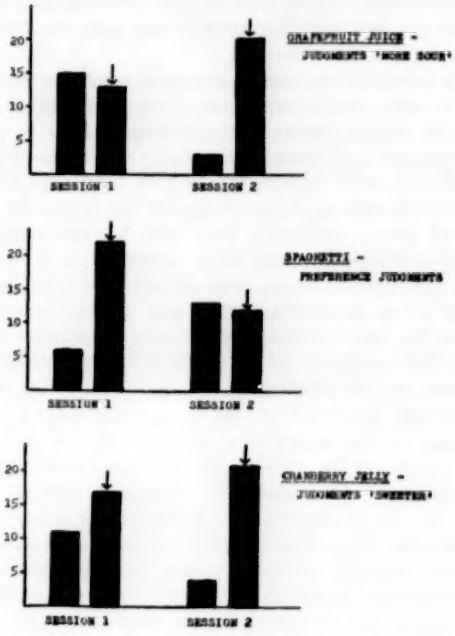

Figure 2. Distribution of observer choices between identical samples under round-table condition.

had no effect upon the responses of the other observers; that is, no significant shift was observed in the direction of the leader's report. In the other three cases, however, the influence of the leader was striking. Differences of 20-3, 21-4, and 22-6 were obtained, all in the same direction as the leader's report, and all significant at better than the .01 level of confidence. Since the pairs of samples were in reality identical, these differences represent a very serious distortion of the true situation.

DISCUSSION

Thus, our hypothesis, that the responses of observers in a round-table situation can be influenced by the report of the first observer, has been confirmed. Although the biassing influence of the round-table environment is not apparent in every case, the results of this brief study have established the existence and all-too-frequent appearance of this hitherto unacknowledged phenomenon.

It has been demonstrated that the round-table effect can create qualitative flavor differences and preferences where none exist. Furthermore, the readily manipulated round-table response results in data of only precarious reliability, since the entire group may be swayed in opposite directions by the report of a single member.

We should indicate, however, that the round-table environment created in this study is not absolute in any sense. Some of the drawbacks which

the present study reveals may to some extent be overcome by well-trained and sophisticated panels; but this in no way detracts from the dangers inherent in the round-table situation.

As previously pointed out, many acceptance-testing environments are but extensions of either the round-table or the individual panel method. The home consumer panel, for example, is closely akin to the round-table method. Samples sent to the home are usually served to the entire family, and attitudes towards and ratings of the test foods are often arrived at through group participation. Thus it should be borne in mind that the biassing effects of group influence may also be operating in situations other than the conventional round-table method.

The present experiment, then, was an attempt to illustrate some of the shortcomings of a frequently used and widely accepted technique. This work, of course, leaves unanswered many problems of methodology; it will have served its purpose, however, if it has established in the minds of some experimenters an awareness of one of the more subtle but vital variables encountered in panel testing.

PYRIDOXINE, INOSITOL AND VITAMIN K CONTENTS OF GERMINATED PULSES

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It was reported earlier from this laboratory that there was increased formation of ascorbic acid *(10)*, thiamine *(7)*, riboflavin *(11)*, carotene *(4)*, choline *(5)*, tocopherol *(6)*, biotin and niacin *(2)* in pulses when they were germinated. While there was not much change in the pantothenic acid content of germinated pulses, folic acid content of germinated pulses diminished greatly during germination *(2)*. Burkholder and McVeigh observed *(3)* a slight increase in the pyridoxine and inositol values in sprouted seeds of *Pisum sativum*, *Phaseolus aureus* and 7 varieties of *Sojax max*. Dam and Glavind *(8)* studied by the biological method the effect of light on the formation of vitamin K in germinated peas and observed that vitamin K content of peas germinated in the presence of light for 16 days was approximately treble the amount of vitamin K of seedlings kept in the darkness for the same duration. Erkama and Pettersson *(9)* determined colorimetrically by means of the redox method vitamin K formation in germinating peas both in the presence and absence of light. The present communication deals with the studies on the pyridoxine, inositol and vitamin K contents of pulses before and after germination for varying periods.

EXPERIMENTAL

Pyridoxine content of pulses. *Germination of the Seeds.* Nearly 1 g. of clean, dry, and healthy looking seeds were weighed and transferred to sterilized petri dishes. Two ml. of glass distilled water was added on the first day and subsequently 1 ml. of water was added daily to each dish. The dishes were kept away from direct sunlight and the seeds allowed to germinate at room temperature (30°C.) for 2 and 4 days.

Estimation of pyridoxine. Powdered ungerminated seeds and seeds germinated for 2 days and 4 days were crushed in a mortar and suspended in 90 ml. of 0.44 N sulfuric acid. The samples were then autoclaved for 1 hour at 20 pounds' pressure, pH was adjusted to 4.5 with 10 N sodium hydroxide using methyl red as external indicator, volume was made up to 250 ml. and filtered. The extract was then diluted in such a way so that the diluted extract contained 20 mμ pyridoxine per ml. Pyridoxine in the extract was estimated microbiologically using *saccharomyces carlsbergensis* (N.C.T.C. 1089) as the test organism according to the method of Atkin, Schultz, Williams, and Frey *(1)*. After 18 hours' incubation at 30°C. the response of the organism was determined turbidimetrically in a Klett-Summerson photoelectric colorimeter using red filter (650 mμ wave length). Pyridoxine contents were calculated by interpolation from the standard curve and expressed in terms of the original weights of the seed before germination. Results are given in Table 1.

Inositol contents of pulses. Seeds were germinated as described in the previous section. Ungerminated seeds were finely ground and seeds germinated for 2 and 4 days were crushed in a mortar, transferred to 100 ml. Erlenmeyer flask with 20 ml. of 1 N sulfuric acid and autoclaved at 15 pounds' pressure for 2 hours, pH was adjusted to 4.5, volume was made up to 100 ml. and filtered. The filtrate was diluted 10 times with water and inositol content was estimated microbiologically using *Sac-*

TABLE 1

Pyridoxine contents of pulses (mg. per 100 g.)

Local name	Botanical name	Ungerminated	After germination	
			2 days	4 days
Khesari	*Lathyrus sativus*	0.65 ± 0.01[1]	0.52 ± 0.01	0.41 ± 0.02
Kalai	*Phascolus mungo*	0.65 ± 0.05	0.87 ± 0.15	0.69 ± 0.03
Matar	*Pisum sativum*	0.14 ± 0.01	0.70 ± 0.06	0.70 ± 0.03
Arhar	*Cajunus indicus*	0.30 ± 0.01	0.41 ± 0.02	0.51 ± 0.03
Mung	*Phascolus radiatus*	0.57 ± 0.03	0.57 ± 0.01	0.79 ± 0.02
Chhola	*Cicer arietinum*	0.54 ± 0.02	0.87 ± 0.05	0.64 ± 0.07
Musur	*Lens esculenta*	0.49 ± 0.01	0.58 ± 0.01	1.13 ± 0.02

[1] Mean and standard error.

charomyces carlsbergensis (N.C.T.C. 1089) in the same way as pyridoxine. Results are given in Table 2.

Vitamin K contents of pulses. Methods of germination of the seeds were the same as described under pyridoxine. The sprouted seeds were rinsed in water, excess water was soaked with blotting paper, dried in an electric oven at 70°C. for 12 hours, weighed and extracted in a Soxhlet apparatus in the dark with petroleum ether boiling at 60°C. for 48 hours. Vitamin K in the ether extract was determined chemically according to

TABLE 2

Inositol contents of pulses (mg. per 100 g.)

Botanical name	Ungerminated	After germination	
		2 days	4 days
Lathyrus sativus	140 ± 10	180 ± 20	200 ± 10
Phascolus mungo	90 ± 0	140 ± 10	170 ± 10
Pisum sativum	150 ± 10	160 ± 10	170 ± 10
Cajunus indicus	100 ± 0	110 ± 10	150 ± 10
Phascolus radiatus	70 ± 0	120 ± 10	110 ± 10
Cicer arietinum	240 ± 10	330 ± 20	330 ± 20
Lens esculenta	130 ± 10	160 ± 10	210 ± 10

the oxidation reduction method of Scudi and Buhs *(12)* which is a colorimetric adaptation of the principle employed by Trenner and Bacher *(13)*, involving a catalytic hydrogenation of the vitamin K-quinone to the hydroquinone stage with Raney nickel catalyst in butanol solution and use of phenosafranine as an indicator. Hydroquinone is reoxidised with 2,6-dichlorophenol indophenol in the absence of air and the decrease in color is measured colorimetrically. Interfering colors present are removed by extracting the hydroquinone after reduction with Claisen's alkali in a special apparatus. The results, expressed in terms of the dry weights of the pulse, are given in Table 3.

TABLE 3

Vitamin K contents of pulses (mg. per 100 g.)

Botanical name	Ungerminated	After germination	
		2 days	4 days
Phascolus mungo	0.13	0.14	0.24
Pisum sativum	0.15	0.18	0.25
Phascolus radiatus	0.30	0.34	0.38
Cicer arietinum	0.29	0.30	0.35
Lens esculenta	0.25	0.29	0.35
Phascolus mungo (Mas kalai)[1]	0.25	0.25	0.34
Cicer arietinum (Kabuli chhola)[1]	0.30	0.32	0.50

[1] Local names.

RESULTS AND DISCUSSION

Pyridoxine values of ungerminated peas *(Pisum sativum)* were lowest of all the varieties of pulses investigated. While pyridoxine content of *Lathyrus sativa* diminished during germination the value increased considerably in the other 6 varieties of pulses studied. Inositol contents of all the 7 varieties of pulses increased during germination. Pulses are good sources of vitamin K. After germination for 4 days vitamin K values increased considerably in all the pulses studied. Germinated pulses, therefore, are better sources of vitamins when compared with ungerminated pulses.

SUMMARY

Pyridoxine and inositol contents of 7 varieties of pulses were estimated microbiologically and vitamin K content was determined colorimetrically before and after germination for 2 and 4 days. With the exception of *Lathyrus sativus* pyridoxine contents of all the pulsose studied increased after germination. Inositol and vitamin K contents of all the pulses increased considerably during germination.

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PALATABILITY AND CHEMICAL DIFFERENCES IN COOKED SMITHFIELD HAMS FROM FULL-FED HOGS ON PEANUT AND CORN RATIONS[a]

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It is traditional to associate Virginia Smithfield hams with peanut-fed hogs. Modern methods of harvesting peanuts and changes in farming practices have greatly reduced the use of hogs to glean peanut fields. Thus, fewer peanut-fed hogs are found on the market.

Fields and Dunker *(7)* studied commercially cured Smithfield hams reported to have come from peanut-fed hogs. These workers described the process as a dry cure, consisting of sodium nitrate, fine salt, and black pepper, with long periods of curing, smoking, and aging. The effect of peanuts and other softening feeds on the physical and chemical properties of pork has been well established, Ellis and Isbell *(6)*. However, palatability and chemical differences in cured Smithfield hams as influenced by ration have not been reported.

This study was planned to determine differences in quality between cured Smithfield hams from peanut- and corn-fed hogs.

EXPERIMENTAL PROCEDURE

Eight pigs (4 of each sex) were selected from a litter and randomized within each sex to two groups. One group of 4 pigs (2 of each sex) was fed a peanut ration which consisted of 71.4% peanuts in the hull and 28.6% corn mixture.[b] The other group was fed a mixed corn ration containing 75% yellow corn, 23.75% supplement,[c] and 1.25% Aurofac-2A (Lederle). After 4 months on these rations the animals were slaughtered and both hams from each pig were tagged and cured along with hams going through the typical Smithfield process.[d] At slaughter, adipose layer was measured at several points over the carcass. The 16 hams were stored almost 2 years.

For the palatability and chemical tests the hams from the 2 lots were paired for size, which gave 8 ham-pairs or tests. The hams were soaked 24 hours in cold water then cooked by simmering[e] in water to an internal temperature of 170.6°F. (77°C.). After cooling in the cooking water, the hams were boned and sliced with a mechanical slicer. Ham weights were recorded at each step of the cooking process.

The ham slices were cut into 1-inch strips for palatability tests. The samples for a particular judge were always taken from the same relative position in the two hams to be compared. The tests included the triangle test of Helm and Trolle *(8)*, the duo-

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[b] Eighty-nine pounds of corn + 3 lb. of mineral mixture + 4 lb. Aurofac-2A (Lederle).

[c] Mixture of two parts tankage and one part alfalfa meal.

[d] By P. D. Gwaltney & Sons, Smithfield, Virginia.

[e] See Committee on Preparation Factors, National Cooperative Meat Investigations *(4)*.

trio test described by Peryam and Swartz *(12)*, and the paired-eating method used by Cover *(5)*.

A panel of 6 judges was selected from a group of volunteers given 16 triangle tests, 8 each of ground and sliced ham samples. A judge was chosen if he could match the 2 *like* samples out of 3 with a precision greater at the 5% level than that expected from chance, 1 in 3.

In the palatability tests the 6 judges scored duplicate samples from the 8 ham-pairs (peanut versus corn ration) which gave 96 observations for each of the 3 palatability tests. Only sliced samples were used for the triangle test since ground samples were not found to increase precision. In the duo-trio test *(12)* the judges matched 1 of 2 unknown samples with a control. Lean tissue was used for this test to determine if the peanut ration gave a characteristic flavor to Smithfield ham. Both tests were carried out in red light which masked appearance differences.

The paired-eating method *(5)* was used to judge the juiciness, tenderness, saltiness, and hardness of lean, desirability of fat layer, general appearance, and sample preference of hams. The chi-square was used to evaluate all palatability tests.

Samples adjacent to the judges' slices were taken for chemical analyses. Dry matter, ether extract, total N, and NaCl were determined on the lean and iodine numbers on the fat by AOAC methods *(2)*. Thiamine was determined by the thiochrome method (AVC) *(14)* using the Fisher nefluorophotometer. Analysis of variance was used to evaluate these data.

From photographs of cross sections of the hams, tracings of fat and lean portions were made on aluminum backed freezer wrapping paper and weighed.

RESULTS AND DISCUSSION

Rate of daily gain of the pigs on the peanut ration was 1.50 lbs., compared to 1.35 lbs. on the corn. Hams from the peanut-fed hogs weighed significantly more than those from the corn-fed hogs both after slaughter and after curing and aging. The former averaged 20.60 and 14.93 lbs. after slaughter and after aging; those from the corn-fed hogs weighed 17.57 and 11.98 lbs., respectively. The loss of weight of the hams during curing and aging was 27.58% (5.67 lbs.) for those from peanut-fed hogs and 31.87% (5.59 lbs.) for hams from corn-fed hogs. The difference in losses was significant on the percentage basis. Total cooking losses were 8.76% (434 g.) for hams from corn-fed animals and 4.17% (269 g.) for those from peanut-fed hogs. These differences were not significant.

The difference in weight of the hams from the peanut- and corn-fed hogs appeared to be due to the difference in amount of adipose fat. Measurement showed the backfat thickness over the hams to be 2.18 inches for peanut-fed and 1.82 inches for corn-fed hogs. The difference, 0.36 ± 0.11 inches, was significant at the 5% level. From the weights of tracings from photographs, these ratios were found: lean, peanut : corn = 0.97 :1, fat, peanut : corn = 1.45 :1. Thus, the amount of lean was approximately the same in the hams from peanut- and corn-fed hogs, but the fat layer was 45% larger in hams from peanut-fed hogs. The lean to fat ratios were 1.55 :1 for the corn-fed pigs and 1.04 :1 for those fed peanuts. The hams from peanut-fed hogs contained as much fat as lean. It must be remembered that these photographs represent one cross section through the center of the ham.

In the triangle tests, fat layers of the hams from peanut-fed hogs were trimmed to equal those from the corn-fed hogs.

The results of the triangle and duo-trio tests are given in Table 1. The judges matched 75 out of 96 triangle tests. This number of matchings was

TABLE 1

The number of correct matchings in the triangle and duo-trio tests of cooked hams from corn- and peanut-fed hogs.

Judge number	Ham-pair[1]								Total score
	1	2	3	4	5	6	7	8	
	No. of correct matchings for triangle test[2]								
1	2	2	1	1	1	1	2	2	12
2	2	2	2	2	2	1	2	2	15
3	1	1	2	1	0	1	1	2	9
4	1	2	2	0	2	1	2	1	11
5	1	2	1	1	1	2	2	2	12
6	2	2	2	2	2	2	2	2	16
Total[3]	9	11	10	7	8	8	11	11	75
	No. of correct matchings for duo-trio test[2]								
1	2	2	1	2	0	0	2	2	11
2	2	2	2	1	1	1	2	2	13
3	2	1	1	2	2	1	2	2	13
4	2	2	2	2	1	1	2	2	14
5	2	2	2	2	2	2	1	2	15
6	2	2	1	2	2	1	1	2	13
Total[4]	12	11	9	11	8	6	10	12	79

[1] Since one ham-pair was lost by spoilage, tests 3 and 4 were taken from the same hams. Tests 3 and 4 were not found to differ statistically from the group.

[2] Two correct matchings were possible for each test.

[3] Chi-square test probabilities were:

	P
Treatment (corn vs. peanuts)	0.001**
Variation among ham-pairs	0.50
Variation among judges	0.10-0.05

[4] Chi-square test probabilities were:

	P
Treatment (corn vs. peanuts)	0.001**
Variation among ham-pairs	0.20-0.10
Variation among judges	0.90-0.80

significantly greater than chance at the 0.1% level. These results showed that, in this study, cooked Smithfield hams from corn- and peanut-fed hogs were judged to be significantly different. In the duo-trio tests the judges matched 79 out of 96 samples correctly. This number again was significantly greater at the 0.1% level than chance matching. These results showed a difference in the lean portion of cooked hams from hogs fed the two rations. Although this test was included to ascertain whether or not the peanut flavor could be identified in the lean portion of the hams, it is recognized that other factors such as saltiness, juiciness, and hardness may have been used by the judge in matching the unknown sample with the control. In both taste tests, variations among the 6 judges or the 8 ham-pairs were not significant.

Palatability factors, ranked by the paired-eating method, differed between hams from peanut- and corn-fed hogs (Table 2). The lean portion of hams from hogs fed peanuts was judged juicier and more tender than that from corn-fed animals, while the latter produced lean ham which the judges found to be saltier and more compact and hard than that from peanut-fed hogs. The fat layer from corn-fed hogs was judged more desirable than that from peanut-fed hogs. Although significant palatability differences were found, the variation among judges was significant in a number of cases. A perusal of the data (Table 2) shows that although an individual judge may differ from the group in judging difference factors, as a whole, the judges were able to detect palatability differences.

TABLE 2

Scores of 6 judges for palatability factors of cooked hams from corn- and peanut-fed hogs.

Palatability factor	Judge						Total	Significance of judge variation
	1	2	3	4	5	6		
	Score for 16 tests[1]							P
Juicier lean								
Corn—	0	3	0	3	2	9	17)	0.02
Peanut—	16	13	16	13	14	7	79)**	
More tender lean								
Corn—	0	3	0	3	2	9	17)	0.02
Peanut—	16	13	16	13	14	7	79)**	
Saltier lean								
Corn—	7	12	16	16	8	13	72)	0.01-0.001
Peanut—	9	4	0	0	8	3	24)**	
More compact, harder lean								
Corn—	16	13	16	13	14	9	81)	0.20-0.10
Peanut—	0	3	0	3	2	7	15)**	
More desirable fat layer								
Corn—	14	16	14	13	11	16	84)	0.50-0.30
Peanut—	2	0	2	3	5	0	12)**	

[1] Duplicate samples from 8 ham-pairs. No significant differences among the 8 ham-pairs were found.

The effect of ration on dry matter, ether extract, and nitrogen content of cooked Smithfield ham was small (Table 3) and seemed to be due to an indirect relationship with the thickness of the adipose layer. Hams from corn-fed animals, which had thinner layers of fat, probably lost more moisture during curing which produced a product higher in dry matter and nitrogen (significant at 5% level).

This difference in thickness of fat layer may also have been responsible to some extent for differences in palatability of hams from corn- and peanut-

TABLE 3

Chemical analyses of cooked hams from corn- and peanut-fed hogs.

Chemical constituent	Corn-fed	Peanut-fed	Difference ± S.E.
	Means of 7 hams		
Lean:			
Dry matter, %	52.41	48.48	3.93 ± 1.45*
Moisture, %	47.59	51.52	3.93 ± 1.45*
Ether extract, %, dry basis	12.30	14.24	1.94 ± 1.11
Ether extract, %, wet basis	6.47	6.91	0.44 ± 0.69
Nitrogen, %, dry basis	10.57	10.38	0.19 ± 0.27
Nitrogen, %, wet basis	5.54	5.03	0.51 ± 0.18*
NaCl, %, dry basis	13.57	13.24	0.33 ± 1.12
NaCl, %, wet basis	7.10	6.39	0.71 ± 0.48
Thiamine, mg. per 100 g., dry basis	1.174	1.796	0.622 ± 0.111**
Thiamine, mg. per 100 g., wet basis	0.614	0.870	0.256 ± 0.053**
Fat:			
Iodine No., Hanus	60.41	88.33	27.92 ± 1.33 **

fed hogs since a greater absorption of salt and loss of moisture could affect juiciness, tenderness, saltiness, and compactness of lean. However, the melting point of the two types of fat produced by the two rations cannot be ignored as an influence on palatability.

Since the palatability tests showed the hams from peanut-fed hogs to be significantly more juicy than those from corn-fed hogs, and since there was significantly more moisture in these hams, a correlation coefficient between these two factors was calculated. This correlation, $r = 0.28$, was not significant at the 5% level. A low correlation (0.31) between juiciness as judged and juiciness as determined by mechanical expression of juice was reported by Satorius and Child *(13)*.

The sodium chloride content (Table 3) of the lean tissue of hams from peanut- and corn-fed hogs did not differ significantly. The palatability tests (Table 2) indicated hams from corn-fed hogs were rated saltier by the judges than hams from peanut-fed hogs. Sodium chloride as analyzed and saltiness as judged were correlated ($r = 0.24$) but the correlation was not significant. Callow *(3)* reports that salt flavor and salt content may not be directly related since the salt within the muscle fibre is probably not as available to the tastebuds as that between the fibres.

The amount of sodium chloride in hams is important. If the amount is too small, spoilage will result; if too large, the hams are unpalatable. Miller and Ziegler *(10)* found that for preservation hams should contain at the bone not less than 9% NaCl, dry basis. This amount was exceeded considerably by the hams in the present study (Table 3). Woodruff, Haynes, and Rodgers *(15)* report that cured hams were scored lower because they were too salty when they contained 5.9% NaCl, wet basis. The hams in the present study contained even more than this amount of NaCl. Thus, it appears that the hams, typical of the Smithfield cure, contained too much NaCl by present standards *(10, 15)*. This excess may have made judging saltiness less critical. It may also explain the low correlation between judges' scores for saltiness and the NaCl content of the hams.

The peanut ration increased significantly the thiamine content of hams (Table 3). When the thiamine content of the two rations was calculated[1] from the composition tables of Morrison *(11)* it was found that the corn and peanut rations supplied 1.54 mg. and 2.40 mg. per lb., respectively. Miller *et al. (9)* reported increases in the thiamine content of fresh pork tissue due to increased thiamine in the ration.

As would be expected, a highly significant difference was found (Table 3) between the iodine numbers of the fat layer of hams from peanut- and corn-fed hogs.

SUMMARY AND CONCLUSIONS

Smithfield hams from peanut- and corn-fed hogs were compared for palatability and chemical composition. Differences in palatability were found between the two groups of hams. The lean portion of the hams from peanut-fed hogs was judged to be more juicy, more tender, less salty, and less compact and hard. Hams from corn-fed hogs were judged to have a

[1] Exclusive of Aurofac-2A (Lederle).

more desirable fat layer. The lean of cooked hams from corn-fed hogs was higher in dry matter and in nitrogen on the wet basis than that from peanut-fed hogs. These differences were thought to be due to a greater loss of moisture during curing, caused by thinner layers of adipose tissue on hams produced on corn ration. The thiamine content of the hams from peanut-fed hogs was greater than that from the corn-fed animals. There was no significant difference in NaCl content. The fat layer of the hams from peanut-fed hogs had a higher iodine number.

Abstract

Smithfield hams from peanut-fed hogs were found to differ significantly in palatability from those from corn-fed hogs. The former were judged more juicy, more tender, less salty, and less compact and hard. Peanut feeding produced a higher thiamine content of the lean tissue and a thicker adipose layer which had a higher iodine number.

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THE EFFECT OF CERTAIN FUNGICIDES ON THE THERMAL DEATH RATE OF SPORES OF *BACILLUS COAGULANS* VAR. *THERMOACIDURANS*

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Despite the rapid increase in the use of fungicides in the control of certain tomato diseases, little research has been done on the various problems arising from their effect on the quality constituents or on the preservation of processed tomatoes.

Bacillus coagulans var. *thermoacidurans* is a spoilage organism that has caused the tomato products industry considerable economic loss. It was thought desirable, therefore, to investigate the effect of several fungicides on the thermal resistance of this organism in a tomato product. Such an effect could be either direct or indirect. The direct effect of these organic spray residues, if they have any, would be upon the spoilage organism *per se*. The indirect effect of these fungicides would be manifested by their possible influence on the pH, total acidity, or soluble and total solids of the product. Changes in these characteristics have been shown to have a pronounced effect on the thermal resistance and the development of *Bacillus coagulans* var. *thermoacidurans (3, 4, 5)*.

The direct type effect could be manifested in several ways. In the tomato field or during the temporary storage of raw tomatoes, such fungicidal residues could control the development of the spoilage organism and thus reduce the initial contamination. Another possibility would be the reverse; namely, these residues might enhance the development of more resistant spores due, in particular, to their cations such as Cu, Zn, or Mn. If some residues are carried over into the can, then a third possibility exists as to the effect of such residues upon the thermal death rate of the contaminating spores and/or the germination and recovery of the spores which survive the thermal process. It is this last possibility which prompted the present investigation.

METHODS AND MATERIALS

Test organism. The culture employed in this study was *Bacillus coagulans* var. *thermoacidurans*, American Type Culture Collection: Strain No. 8038.

Preparation of spore suspension. In the course of an investigation on the sporulation properties of the test organism, a tomato juice agar (containing 10% tomato juice, 0.5% KH_2PO_4, 2% agar, and adjusted to pH 5) was found to support a more rapid and abundant spore crop than did several other laboratory media, including the classically used proteose peptone acid agar.[b] Therefore, tomato juice agar was employed for the production of spores.

Agar slants were prepared in 16 oz. prescription bottles and inoculated with a 24-hour proteose peptone broth culture. Incubation was at 113°F. (45°C.) for 7 days. Cold distilled water was used to harvest and to wash the spore crop. After each

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[b] Thermoacidurans agar—Difco.

centrifugation, the suspension was blended in a micro Waring blender for 2-5 minutes. After 4 washings this procedure provided a spore suspension almost free of vegetative cells. The washed spores were suspended in M/40 KH_2PO_4-NaOH buffer and stored under refrigeration in a screw-cap 8 oz. prescription bottle containing about one-half inch No. 2 glass beads. The spore count of the stock spore suspension was determined by pasteurizing a 3 ml. aliquot in a water bath at 199.4°F. (93°C.) for 5 minutes and plating on thermoacidurans (Difco) agar containing 0.1% soluble starch. Incubation was at 113°F. (45°C.) and the plates were counted at the end of 5 days.

Preparation of the heating menstruum. Normal tomato juice of pH 4.2 was used as the basal heating menstruum for the thermal death rate studies. The juice was prepared in the conventional manner from an unsprayed single lot of sound fresh tomatoes. The finished juice was filled into No. 2 plain tin sanitary type cans which were then sealed and sharp-frozen overnight at –40°F. (–40°C.). The following day the frozen product was transferred to 0°F. (–17.8°C.), where it was stored until used. As needed, a can of the frozen juice was removed from storage and thawed quickly in the Arnold steamer. For each thermal death rate determination a 300 ml. aliquot of the defrosted juice was transferred into the reaction chamber of the thermal death rate apparatus.

Method of determining the thermal death rates. The apparatus used was similar to that used by Malin *(2)* and Kaufmann and Andrews *(1)*.

The procedure used for determining the thermal death rates was as follows: A sufficient amount of the fungicide to give a final concentration of 100 p.p.m. active material was weighed out and introduced into the reaction chamber. A 300 ml. aliquot of the defrosted and warmed tomato juice was then added and the reaction chamber tightly sealed and suspended in the secondary heating bath at the proper level. The primary heating bath had been previously heated to the predetermined temperature, and circulation had been started. The Magna Stir was then turned on to a preset level to keep the tomato juice adequately stirred. The potentiometer was checked at close intervals until a constant reading was maintained for at least 10 minutes. In the meantime, under continuous heating and stirring, the fungicide had adequate opportunity to be uniformly suspended or dissolved in the tomato juice. As soon as the temperature equilibrium was indicated, the death rate determination was begun. To check on the sterility of the heated juice, a sample was first withdrawn from the hot juice, cooled, and plated. The stock spore suspension, already adjusted to give a spore concentration of about 10^9 per ml., was shaken vigorously for 5 minutes to break up possible clumps. At zero time, one ml. of the shaken spore suspension was injected well below the surface of the heating menstruum via one of the sampling vents and through the rubber diaphragm. At the end of the first 5 minute heating period, a sample was withdrawn to give the initial spore count in the heating menstruum. Next, samples were drawn at predecided intervals. Any withdrawn sample was very rapidly discharged into a cold sterile culture tube and immediately chilled in an ice bath. Cooled samples were plated in triplicate on thermoacidurans agar containing 0.1% soluble starch. Plates were incubated for at least 5 days at 113°F. (45°C.) before final counts were made.

Fungicides tested.[a] The following is a list of the fungicides tested and their respective percentages of active ingredients.

Assigned name	*Chemical name*	*% active ingredients*
Fixed copper	Tribasic copper sulfate	53
Zeneb	Zinc ethylene bisdithiocarbamate	84.6
Maneb	Manganese ethylene bisdithiocarbamate	70
Captan	N-trichloromethylthiotetrahydrophthalimide	50
Dichlone	2,3-dichloro-1, 4-naphthoquinone	60

All of the foregoing fungicides are water insoluble but apparently somewhat soluble in tomato juice. Duplicate thermal death rate determinations were made in the case

[a] These fungicides were made available through the courtesy of Dr. M. B. Linn, Department of Horticulture, University of Illinois.

of each fungicide. The average death rate in each case was then compared with that of the control where no fungicide was added to the tomato juice.

Statistical treatment of thermal death rate data. All sets of triplicate plate counts were examined and found in most cases to be distributed in accord with the Poisson distribution (i.e., mean and variance were in reasonable agreement). The linear regression of the logarithms of the mean plate counts on the time intervals was fitted in each thermal death run, using the method of "least squares." Duplicate values of the regression coefficient were averaged, using proper statistical weights. The difference between the average value of the regression coefficient for each fungicide and that for the no-fungicide control was statistically examined using the t test and the level of significance was accordingly established. The D values were also computed from the corresponding regression coefficients.

RESULTS AND DISCUSSION

The duplicate values of the thermal death rate constant D and their statistically weighed means are presented in Table 1. The table also includes

TABLE 1

The effect of several fungicides[1] on the thermal death rate of spores[2] of *B. coagulans* var. *thermoacidurans*

Treatment with fungicide	D values[3]		Weighed mean of D	Observed t[4]	Tabulated P
	Run a	Run b			
No fungicide	11.15	9.31	10.41	.....	.....
Fixed copper	5.44	5.73	5.65	8.71	0.01−
Zeneb	9.06	9.07	9.07	1.81	0.1+
Maneb	7.81	7.96	7.89	2.62	0.05−
Captan	8.09	7.36	7.71	5.25	0.01−
Dichlone	8.40	8.17	8.24	2.72	0.05−

[1] 100 p.p.m. active ingredient.
[2] About 10^7 spores per ml., heated at 192.2°F. (89°C.) in tomato juice of pH 4.2.
[3] D = minutes required for a 90% reduction in the number of survivors.
[4] Sum of squares of the difference between duplicate regression coefficients = 0.287, and the mean square = 0.047. Tabulated values of t are: 2.447 at the 0.05 level and 3.707 at the 0.01 level for 6 degrees of freedom (difference between duplicate runs).

the statistical t and P of the difference between the mean coefficient obtained with each fungicide and that of the no-fungicide control. It is evident from Table 1 that all the fungicides tested, except Zeneb, enhanced the thermal death rate significantly.

The inhibitory action of the fungicides. During the course of the thermal death rate determinations just described an interesting phenomenon was observed. In every case when fungicides were added to the heating menstruum, and when undiluted or 10^{-1} dilution plates were made at the late heating intervals, no colonies developed on the plates. These plates, according to the extrapolated survivor curves should have had countable numbers of colonies. This phenomenon was tentatively interpreted as an inhibitory action of the fungicides. Inhibition was not manifested during the heating process of the earlier intervals because the samples were diluted to a noninhibitory level before plating. At zero and 10^{-1} dilutions there was enough residue of the fungicide carried over to the plate to inhibit germination and/or the development of colonies.

To check the above interpretation the following experiment was carried out: Six short-time thermal death runs were made in exactly the same manner as previously described. In the first run no fungicide was added

to the heating menstruum whereas in the remaining 5 runs the 5 fungicides were added, one at a time, to give a final concentration of 1000 p.p.m. As soon as the temperature equilibrium was reached a sterility sample was withdrawn. At zero time, one ml. of the spore suspension, adjusted to give an average count of about 10^6 spores per ml. of juice, was injected. At the end of 5 minutes a sample was withdrawn and the run was ended to start the next one. Undiluted, 10^{-1}, 10^{-2}, and 10^{-3} dilutions were made up from the 5-minute samples and were plated in triplicate, using exactly 20 ml. of thermoacidurans agar containing soluble starch. This procedure resulted in a final concentration of the fungicide, carried over to the plates, of 50, 5, 0.5, and 0.05 p.p.m., respectively. Plates were incubated at 113°F. (45°C.) for 5 days, examined, and then stored at room temperature for four more weeks before a final examination was made.

Results of the experiment are presented in Table 2.

TABLE 2

The inhibitory effect of residual fungicides against Bacillus coagulans var. thermoacidurans

Dilutions plated[1]	0	10^{-1}	10^{-2}	10^{-3}
Calculated residual fungicide in plate (PPM)[2]	50	5	0.5	0.05
	Colonies per plate			
Calculated	1,000,000	100,000	10,000	1,000
Observed:				
No fungicide	TNTC[3]	TNTC	TNTC	TNTC
Fixed copper	0	7	450	TNTC
Zeneb	0	TNTC	TNTC	TNTC
Maneb	0	0	456	TNTC
Captan	0	3	TNTC	TNTC
Dichlone	0	0	251	TNTC

[1] Tomato juice containing 1000 p.p.m. of fungicide was heated to 192.2°F. (89°C.), inoculated to a count of 10^6 spores per ml. of juice, held at that temperature for 5 minutes and then sampled. Triplicate plates were made for the dilutions indicated.

[2] The concentrations indicated, assume that the fungicide (1000 p.p.m.) in the heated tomato juice sample was uniformly dispersed during sampling and dilution for plating. As each plate contained 20 ml. of agar medium, the figures given represent the calculated concentration of residual fungicide transferred to each plate.

[3] Too numerous to count.

From Table 2 it can be seen that all the fungicides tested exhibited some inhibitory action. With all the fungicides the 50 p.p.m. level inhibited growth completely for at least 33 days. With all except Zeneb, the 5 p.p.m. concentration was also effective. At the 0.5 p.p.m. level, however, two fungicides (Zeneb and Captan) did not show an inhibitory effect, whereas the remaining three (Fixed copper, Maneb, and Dichlone) still exhibited partial inhibition. All the fungicides failed to show an inhibitory effect at the 0.05 p.p.m. level.

These results explain the no-growth phenomenon encountered in the previous thermal death rate determinations. In the undiluted and 10^{-1} dilution plates the fungicide's level was about 5 and 0.5 p.p.m. respectively. The examination of individual data showed that Zeneb and Captan were less inhibitory. It also showed no inhibition beyond the 10^{-1} dilution. The apparently higher inhibitory effect noticed during the original thermal death rate determinations might have been due to the much longer heating

process and/or a reduced volume of recovery medium added to the recovery plates.

These results demonstrate a definite and a direct influence of the fungicides tested upon both the thermal resistance and the recovery of heated spores of the test organism. However, further evaluation of the fungicidal action under conditions similar to those which prevail in the commercial operation is necessary before final conclusions can be drawn.

The most probable magnitude of such fungicidal residues, carried over into the final product, should be established. More representative strains, the spores of which are virulent enough to recover and initiate typical flat sour spoilage in the processed cans of tomato juice, must also be found. Once such information and material is made available, inoculated pack experiments using different strains and the proper concentrations of the fungicides, can then be undertaken in order to confirm the laboratory results. Adequate replication must also be considered to validate statistical analysis of data.

Another type of influence is also worthy of investigation, namely; the influence which such organic residues might exert upon the growth cycle of the test organism. Specifically, it would be of much interest to ascertain their effect on its sporulation ability and the thermal resistance of the spores produced. Furthermore, the influence of these fungicides upon the quality constituents of tomatoes, which in turn affect the thermal resistance of the spores, should be investigated. Work is now underway to evaluate the potentiality of these possible fungicidal influences. Results will be reported in later publications.

SUMMARY AND CONCLUSIONS

The effect of several fungicides on the thermal resistance of spores of *Bacillus coagulans* var. *thermoacidurans* was investigated. Under laboratory conditions 100 p.p.m. of Fixed copper, Maneb, Captan and Dichlone, in tomato juice of pH 4.2 caused a significant decrease in the D value of the thermal death rate. The fungicides were also demonstrated to have a sporostatic effect at the concentrations of 50, 5, and 0.5 p.p.m. in thermoacidurans agar plates.

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EFFECT OF SKIN COATINGS ON THE STORAGE BEHAVIOUR OF MANGOES

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Recent research has shown that skin coatings of various types may increase considerably the storage life of fruits and vegetables in non-refrigerated storage. Hall, Sykes, and Trout *(5)* recorded an increase of about 50% in the storage life of Granny Smith apples in non-refrigerated storage with the aid of an application of 8-10% alcoholic solution containing 2 parts of castor oil and one part of wax-free shellac. Bose and Basu *(2)* have used wax coatings in an attempt to increase the storage life of "fajli" mangoes.

In the United States, the waxes generally employed commercially for prolonging the storage life of fresh fruit are paraffin-carnauba mixtures containing approximately 7% solids. The fruits to be waxed are passed along on a conveyor belt or on rollers beneath nozzles which drip the wax emulsion continuously on the products. After undergoing waxing, the produce may pass either through a tunnel into which warm air is blown or to a receiving receptacle where it may be allowed to dry naturally *(3)*.

In England, a 12% emulsion of equal parts of peanut oil and medicinal paraffin oil has given satisfactory results as a coating for fruits in non-refrigerated storage *(7)*.

In the present investigation the effects of two skin coatings on the storage behaviour of mangoes were studied. The coatings studied were a wax emulsion in water and a refined mineral oil. Three varieties of mango —Padre, Neelam, and Totapuri—were used in the experiments.

MATERIALS AND METHODS

The fruits for this investigation were obtained direct from the orchards and were fully grown and green, corresponding to Stage B (the shoulders outgrow the stem end and the colour of the fruit is oil green) of Cheema and Dani *(4)* and stage 2 (shoulders are raised above the hollow in which the stem end is inserted) of Wardlaw and Leonard *(9)*. The fruits were dipped in a paraffin wax emulsion in water containing approximately 7% solids. In the case of the refined mineral oil, the treatment was given by rubbing a piece of cotton wool previously soaked in the oil over the whole surface of the mango. In a later experiment, specified parts of the mango were treated with the mineral oil in the same manner. The fruits were dried at room temperature and stored at room temperature (72-82°F.; R.H., 65-90%).

Respiration rates were measured at 67-70°F. by the continuous-current method, the CO_2 evolved being absorbed in N/10 barium hydroxide solution. The barium hydroxide was subsequently titrated against standard hydrochloric acid. For the estimation of ascorbic acid, 10 g. of the edible portion were blended in 4% metaphosphoric acid solution and filtered and the filtrate was titrated against a standard solution of 2:6 dichlorophenol indophenol. For the determination of total acidity, 10 g. of the edible portion were macerated in a blender with distilled water and filtered. The filtrate was titrated against N/10 NaOH. Total soluble solids were determined by means of a hand refractometer. Reducing sugars were estimated by the Munson-Walker-Bertrand Method *(6)*. Moisture contents were determined by drying 10 g. of the edible portion at 75°C. for 16 hours.

RESULTS AND DISCUSSION

Data concerning the percentage physiological losses in weight, i.e., losses due to respiration and transpiration in treated and untreated mangoes, are presented in Table 1. The physiological losses in weight were

TABLE 1

Percentage physiological losses in weight in treated and untreated mangoes during storage.

Variety	Treatment	Original wt., grams	Percent losses in weight after		
			7 days	10 days	14 days
(1) Padre	Mineral oil	836.2	3.3	4.1	....
	Wax emulsion	691.7	2.3	2.9	....
	Control	775.7	8.4	10.5	....
(2) Neelam	Mineral oil	653.0	1.9	5.0	....
	Wax emulsion	705.0	1.8	4.8	....
	Control	577.5	3.2	5.7	....
(3) Totapuri	Mineral oil	1136.0	2.4	....	5.8
	Wax emulsion	1346.5	1.3	....	2.8
	Control	1232.5	3.6	....	7.1

found to be the least in the case of waxed mangoes followed by those that had received a coating of mineral oil and by the controls. Table 2 shows that treatment with mineral oil depresses respiratory activity to a greater degree than does the wax treatment. It follows, therefore, that waxing acts mainly to control losses of moisture. It controls respiration rate also to some extent, whereas oil treatment controls mainly the respiration rate. Losses of moisture are controlled to a lesser degree by the latter treatment. The climacteric rise in the respiration characteristic of senescent fruits was also recorded in the 3 varieties of mango investigated *(1, 8)*. Data concerning certain chemical changes in treated and untreated mangoes are presented in Table 3. Decreases in ascorbic acid contents and acidity and increases in the contents of total soluble solids and reducing sugars were observed both on fresh weight as well as original fresh weight bases in all the varieties and in all the treatments. The moisture contents also decreased in all the varieties and in all the treatments at the end of the storage period.

TABLE 2

Respiration rates in treated and untreated mangoes during storage.

Variety	Treatment	Initial resp. rate	Respiration rate after		
			7 days	10 days	14 days
			mg. CO_2/kg./hr.		
(1) Padre	Mineral oil	24.5	.....	29.0	.....
	Wax emulsion	24.5	.....	56.4	.....
	Control	24.5	.....	103.8	.....
(2) Neelam	Mineral oil	20.1	42.7	22.8	.....
	Wax emulsion	20.1	47.1	27.1	.....
	Control	20.1	67.7	54.5	.....
(3) Totapuri	Mineral oil	30.1	38.8	.....	24.3
	Wax emulsion	30.1	41.1	.....	24.9
	Control	30.1	44.1	.....	39.4

TABLE 3

Chemical changes in treated and untreated mangoes during storage (fresh weight basis). The data in parentheses represent values calculated on original fresh weight basis at the end of the storage period.

Variety	Treatment	Ascorbic acid (mg./100 gm)		Acidity as malic acid, %		Total soluble solids, %		Reducing sugars %		Moisture %	
		C[1]	E[2]	C	E	C	E	C	E	C	E
(1) Padre[3]	Mineral oil	24.1	20.6 (19.6)	1.8	0.42 (0.40)	16.0	19.5 (18.5)	2.4	3.2 (3.0)	83.0	78.4
	Wax emulsion	24.1	20.2 (18.6)	1.8	0.23 (0.21)	16.0	18.6 (17.1)	2.4	3.9 (3.6)	83.0	76.2
	Control	24.1	20.0 (18.0)	1.8	0.17 (0.15)	16.0	18.9 (17.0)	2.4	3.5 (3.2)	83.0	74.7
(2) Neelam[3]	Mineral oil	58.8	42.0 (40.7)	0.46	0.23 (0.22)	18.0	20.8 (20.2)	3.7	5.4 (5.2)	77.5	75.0
	Wax emulsion	58.8	38.1 (37.7)	0.46	0.15 (0.15)	18.0	21.0 (20.8)	3.7	5.7 (5.6)	77.5	77.0
	Control	58.8	52.0 (47.8)	0.46	0.15 (0.14)	18.0	21.0 (19.3)	3.7	5.4 (5.0)	77.5	71.1
(3) Totapuri[4]	Mineral oil	26.8	14.0 (13.7)	1.0	0.70 (0.67)	12.0	16.0 (15.7)	3.1	4.6 (4.5)	78.2	76.5
	Wax emulsion	26.8	12.2 (11.3)	1.0	0.18 (0.17)	12.0	16.0 (14.8)	3.1	4.8 (4.5)	78.2	73.0
	Control	26.8	15.0 (14.3)	1.0	0.11 (0.10)	12.0	17.0 (16.1)	3.1	4.4 (4.2)	78.2	74.2

[1] C signifies commencement of storage.
[2] E indicates the end of storage.
[3] The storage period was 10 days.
[4] The storage period was 14 days.

TABLE 4

Storage life of treated and untreated mangoes on the basis of 10% wastage.

Variety	Treatment	Storage life, days
(1) Padre	Mineral oil	7
	Wax emulsion	9
	Control	6
(2) Neelam	Mineral oil	8
	Wax emulsion	10
	Control	7
(3) Totapuri	Mineral oil	9
	Wax emulsion	12
	Control	8

Data concerning the storage lives on a 10% wastage basis of treated and untreated mangoes at the room temperature (72-82°F.; R.H., 65-90%) are recorded in Table 4. Both the treatments, viz., wax and oil, were found to increase the storage life of all three of the varieties of mango investigated. Wax treatment was found to increase the storage life of mango to a greater extent than did the oil treatment. Increases in storage life of the order of about 50% were generally obtained with the wax treatment.

A fairly large number of mangoes in the oil-treated lot were found at the end of the storage period to suffer from a functional disease involving the whole skin of the fruit. A typical case is shown in Figure 1. This oil injury produced a bitter taste in the fruit.

As no organism was found associated with the disease, it was considered that the disease might have resulted from an impairment of normal gaseous exchange through the skin, leading to anaerobiosis and culminating in the skin blemishes observed. To test this theory, another experiment was conducted with Neelam mangoes only wherein the mineral oil was applied to specific parts of the fruit, leaving part of the skin free to

Figure 1. A typical case of mineral oil injury in Totapuri Mango.

Figure 2. Neelam mangoes at the end of 13 days' storage from the experiment in which mineral oil was applied to specific parts of the skin only. Mangoes belonging to treatment 1 (see Fig. 3) were found in an excellent condition, others were found affected with various diseases.

carry no normal respiration. The details of this application of oil are shown in Figure 3. In treatments 1, 2 and 3 a longitudinal axis was assumed in the middle of the fruit and parts of the skin corresponding to one-third of this axis were treated with mineral oil. The parts of the fruit that received the mineral oil treatment have been shaded in Figure 3.

Data on the physiological losses in weight in Neelam mangoes treated with mineral oil to various extents are recorded in Table 5. These losses were highest in untreated mangoes and least in mangoes that had been completely covered with the mineral oil. Losses in weight in the mangoes

TABLE 5

Percentage physiological losses in weight in Neelam mangoes treated with mineral oil to various extents.

Treatment No.	Original weight of 2 mangoes	Percent losses (days)				
		4	6	8	10	13
1	318.0	0.6	1.0	1.5	2.3	4.0
2	291.2	0.5	1.0	1.5	....	....
3	284.0	0.5	0.7	1.1	....	....
4	333.4	0.4	0.5	1.0	....	....
5	288.4	0.7	1.1	1.7	....	....

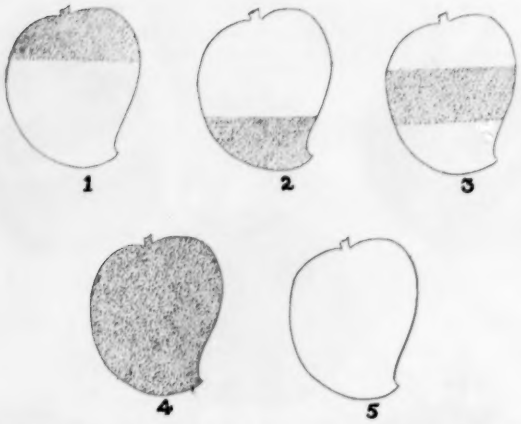

Figure 3. Neelam mangoes. The shaded parts were treated with mineral oil.

that had been partly treated with mineral oil were intermediate between these two extremes. The respiration rates in Neelam mangoes treated with mineral oil to various extents are graphically shown in Figure 4. The rate of respiration was highest in the untreated mangoes and lowest in the completely treated mangoes. Among the partly treated mangoes, the rate of respiration was the lowest in treatment 1—i.e., where only the top one-third of the fruit was treated with the mineral oil. This shows that normally the stem end of the mango has the highest respiration rate. All 5 respiration curves in Figure 4 show the climacteric rises characteristic of senescent fruits *(1, 8)*. Mangoes that had received the mineral oil treatment on the top one-third only showed an increase of over 50% in storage life as compared to the controls. Figure 2 shows the condition of the treated Neelam mangoes and the control at the end of 13 days of storage at room temperature (72-82°F., R.H. 65-90%). Mangoes with the top one-third treated with mineral oil were found to be in excellent condition. Mineral oil treatment of only the top one-third of the fruit is therefore a considerable improvement over the technique of covering the whole surface of mango with mineral oil.

SUMMARY

The comparative effect of two skin coatings, viz., a wax emulsion in water and a refined mineral oil, on the storage behaviour at room temperature (72-82°F.; R.H. 65-90%) of 3 varieties of mango—Padre, Neelam, and Totapuri—has been studied.

The physiological losses in weight, i.e., losses due to respiration and transpiration—were found to be least in the case of waxed mangoes, followed by the mineral oil treated mangoes and finally the controls. Treatment with oil depressed respiratory activity to a greater extent than did the wax coating. During storage, decreases in ascorbic acid content and acidity and increases in the content of total soluble solids and reducing

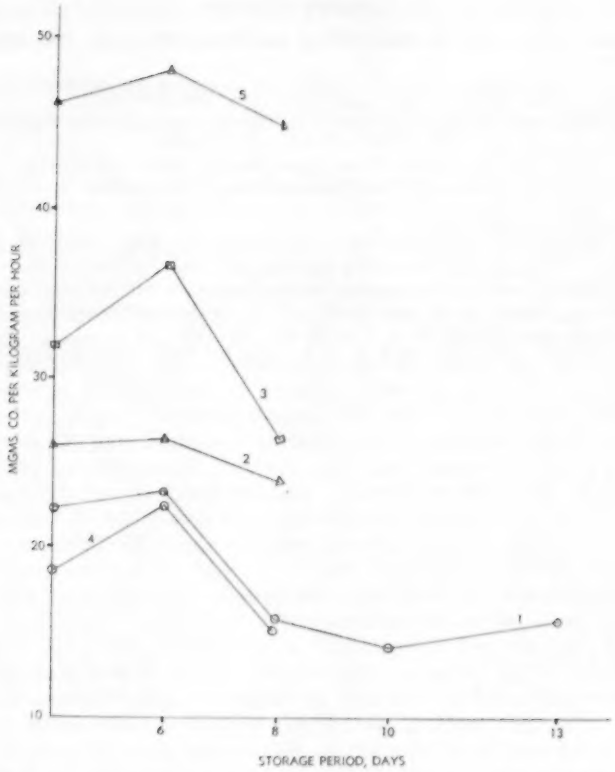

Figure 4. Respiration rates during storage of Neelam mangoes treated with mineral oil to various extents (For explanation of numbers, see Fig. 3).

sugars were observed in all the varieties of mango and in all the treatments investigated.

With a coating of wax emulsion in water, increases in storage life at room temperature (72-82°F.; R.H., 65-90%) of the order of about 50% were obtained in all the varieties investigated. Application of a mineral oil coating to the whole surface of mangoes did not increase the storage life to the same extent as wax coating. Moreover, application of the mineral oil to the whole surface of the mangoes resulted in the production of an oil injury on the skin of the fruits. Mangoes in which only the top one-third was treated with mineral oil remained free from the oil injury and storage life was increased by over 50%.

Acknowledgment

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ANALYTICAL CHARACTERISTICS OF PROPENYL GUAETHOL AND VANILLIN

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For flavoring purposes, numerous products have been proposed to supplement or substitute for the natural extractives from the vanilla bean. Because coumarin has recently been pronounced toxic, and its use in flavoring extracts forbidden by the U. S. Food and Drug Administration, new products to extend the nature and variety of flavoring materials are now of greater interest. One synthetic compound which has shown promise and has attracted attention is propenyl guaethol, known under the trade name of Vanitrope [1 hydroxy 2 ethoxy *(4)* propenyl benbene]. Cartwright and Kelley *(3)* have evaluated its flavor quality and strength in comparison to vanillin and associated chemicals, and have assigned a factor of twenty times vanillin for its flavor strength. Further study of its properties by Cartwright *(2)* has shown that its solubility in water is very low—only 0.002% at 25°C.—so that a solution in water is much too weak for use as an ordinary extract. In 50% alcohol the solubility is 0.54%, which compares very favorably with that of vanillin. However, regulations relative to the use of ethyl alcohol make this solvent less desirable. Propylene glycol with 50% water can dissolve 0.11% Vanitrope, and in the pure glycol the solubility is increased to 4%. Accordingly propylene glycol has been employed frequently in the preparation of extracts. The use of polyoxyethylene sorbitan monooleate has also been suggested by Tyler *(9)*.

Comparatively little information on the analysis of vanitrope is available. Roberts *(8)* has proposed a method for its determination in the presence of other materials frequently found in imitation vanilla extracts. The method is based upon a preliminary separation of the vanitrope by a steam distillation, after which the separated vanitrope is coupled with p-nitrobenzenediazonium chloride and the resulting product is estimated colorimetrically. The method is somewhat involved and other simpler procedures are desirable. Since it is usually proposed that the vanitrope be used along with vanillin in the preparation of flavors, knowledge of its conduct in the various procedures used to determine vanillin is essential. To secure information of this nature and develop methods for its analysis was the object of this study.

COLORIMETRIC PROCEDURE

The modified Folin-Denis colorimetric method *(1)* which involves the reduction of a phosphomolybdic-phosphotungstic acid mixture is official (A.O.A.C.) for vanillin. Since the reaction is characteristic of phenols it would be expected that vanitrope would react but the rate and extent of the reaction could differ from vanillin because of the differences in the nature of the substituted groups on the benzene ring. It has been shown

(6) that some of the difficulties in the earlier modification of the colorimetric method can be lessened by use of a lower concentration of sodium carbonate and inclusion of some sodium metaphosphate in the solution. In the present experiments, in which the reducing action of vanillin and vanitrope was compared, the conditions were in accordance with these improvements.

Equipment. A photoelectric colorimeter, Lumetron Model 400 A, equipped with a red filter which transmitted a wave band of mean wave length 650 mμ was employed.

Reagents. Folin-Denis reagent: Prepared according to the Methods of the A.O.A.C. (1).

Sodium carbonate: 11 g. per 100 ml.

Sodium metaphosphate: 10 g. per 100 ml.

Stock Vanillin solution: 1.000 g. vanillin dissolved in 250 ml. 95% ethyl alcohol and diluted to one liter with water.

Stock Vanitrope solution: 1.000 g. vanitrope dissolved in 95% ethyl alcohol and diluted to one liter with alcohol.

Diluted Standard Vanillin solution: 10 ml. stock vanillin pipetted into a 100 ml. volumetric flask and diluted to volume. Each ml. = 0.1 mg. vanillin.

Diluted Standard Vanitrope: Prepared similarly from stock vanitrope. Each ml. = 0.1 mg. vanitrope.

Measured portions of the standard solutions were taken to give the quantities specified in Table 1.

PROCEDURE

The quantities of standard vanillin, or vanitrope, indicated in Table 1 were introduced into 100 ml. volumetric flasks. To each flask, 5 ml. of the sodium metaphosphate solution was added and then 5 ml. of Folin-Denis reagent. After standing for 5 minutes, 10 ml. of the sodium carbonate solution was added to each flask and after an additional 15 minutes, each solution was made to volume with distilled water and mixed thoroughly. The color values were measured with the photoelectric colorimeter.

RESULTS

The results are given in Table 1.

TABLE 1

Color values resulting from the reaction of vanillin and vanitrope in modified Folin-Denis procedure

	Vanillin				Vanitrope			
mg.	0.1	0.3	0.5	0.8	0.1	0.3	0.5	0.8
Time of standing min.			Absorbancy × 10					
15	1.05	2.41	3.49	4.30	1.35	3.87	5.26	6.84
20	1.05	2.48	3.49	4.30	1.40	3.90	5.35	6.95
25	1.05	2.50	3.52	4.37	1.40	3.92	5.38	7.00
30	1.05	2.50	3.57	4.42	1.42	3.98	5.40	7.05
40	1.07	2.52	3.64	4.55	1.42	4.00	5.52	7.15
50	1.09	2.58	3.68	4.60	1.42	4.10	5.62	7.25
60	1.10	2.59	3.70	4.65	1.44	4.10	5.62	7.18
75	1.10	2.63	3.77	4.73	1.44	4.18	5.75	7.40
90	1.10	2.65	3.86	4.81	1.46	4.21	5.80	7.55
Hours								
in light 24	1.28	3.05	4.40	5.45	1.73	4.85	6.65	9.10
in dark 24	1.26	2.97	4.49	5.40	1.73	4.85	6.65	9.10

DISCUSSION

The fact that both vanillin and vanitrope have pronounced reducing action on the Folin-Denis reagent demonstrates that the colorimetric method alone will not be satisfactory for the determination of vanillin in the presence of vanitrope. However, it is likely that a combination of the procedure with some other method by which one of the two constituents can be determined directly may make possible a calculation of the absorbancy due to one and an estimation of the other constituent from the residual absorbancy. As shown in the table, the absorbancy for each compound increases with time of standing and at about the same rate. Hence, there is no contribution to differentiation on this basis. Other experiments, not included, have demonstrated that a change in the concentration of sodium carbonate used in the Folin-Denis procedure from a 10% to a saturated solution alters the reducing action very slightly. It makes very little difference whether the reaction proceeds in the dark or under regular laboratory illumination.

Ultraviolet Absorption

In 1944 Englis and Hanahan *(5)* presented a method for the analysis of flavoring extracts containing vanillin and coumarin in which an evaluation of the quantity of each was made from the absorbance of a neutral, or slightly acid, solution at two selected wave lengths in the ultraviolet region. Later work by Lemon *(7)* has shown that when a vanillin solution is made alkaline, a peak of maximum absorption appears at a much longer wave length. Furthermore, the absorbance is increased and the peak is in a region less subject to interference by coumarin. Emsinger *(4)* has made application of ultraviolet absorption spectra to the examination of more complex mixtures of this nature. No data have been found for the ultraviolet spectrum of vanitrope and knowledge of its characteristics in both acid and basic solutions is essential.

Equipment

A Cary recording spectrophotometer Model 11.
Beckman pH meter, Industrial Model.

Reagents

$NaH_2PO_4 \cdot H_2O$, 50 g. per liter
$Na_2HPO_4 \cdot 12\ H_2O$, 100 g. per liter
Standard Vanillin
Standard Vanitrope

PROCEDURE

Aliquots of the standard vanillin and vanitrope solutions were measured into volumetric flasks and a 10 ml. portion of the appropriate phosphate solution was added to each before making to volume. The monosodium phosphate was used as a buffer for the acid range and the disodium phosphate as a buffer for the alkaline range. The quantities of the various reagents used are indicated in Table 2. The absorption values in the range 200-400 mμ were measured against a blank containing the reagents as the reference solution. Representative curves are shown in Figures 1 and 2.

Published data shows that the absorbancy of vanillin in acid solution near 232 and 280 mμ *(5)* and in basic solution near 347 mμ *(7)* follows Beer's Law. In the present work, the same fact was noted for vanitrope at selected wave lengths. In Table 2 are given the absorbancies for both compounds under the conditions of operation. For sake of completeness of record, values are given for vanillin at its various maxima,

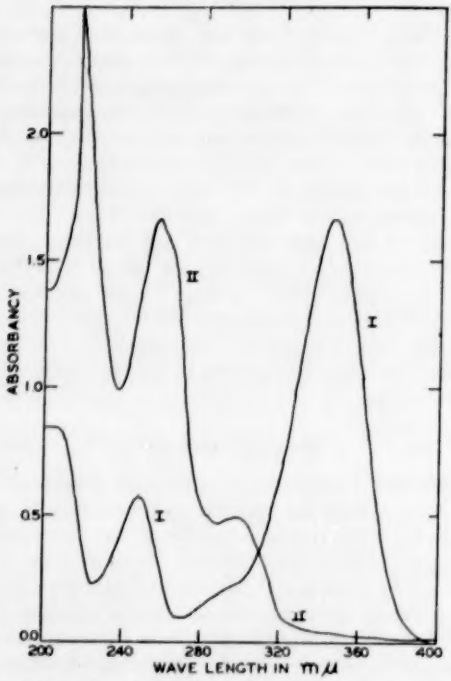

Figure 1. Absorption curves for vanillin and vanitrope in basic solution (pH = 9). I 10 mg. vanillin per liter. II 20 mg. vanitrope per liter.

229, 279, and 308 mμ in acid solution, as well as the other values actually employed in later analytical procedures.

Analysis of vanillin and vanitrope mixtures from absorbancy values of a basic solution. Employing ultraviolet absorption data, several methods of procedure for the analysis of mixtures of vanillin and vanitrope are possible. In Figure 1 it is shown that at pH 9 the absorption of vanillin at λ 347 mμ is at a maximum, while vanitrope has practically a negligible absorption. Hence, if the ratio of vanitrope to vanillin is small, an estimate of the latter may be made directly from the absorbance at 347 mμ.

TABLE 2

Absorbancy values for vanillin and vanitrope concentration 10 mg. per liter

		Vanillin	Vanitrope
	λ in mμ	A	A
pH of solution			
9	347	1.67	0.02
9	256	0.42	0.815
4.6	256	0.21	0.865
4.6	295	0.555	0.245
4.6	229	0.925	.....
4.6	279	0.675	.....
4.6	308	0.605	.....

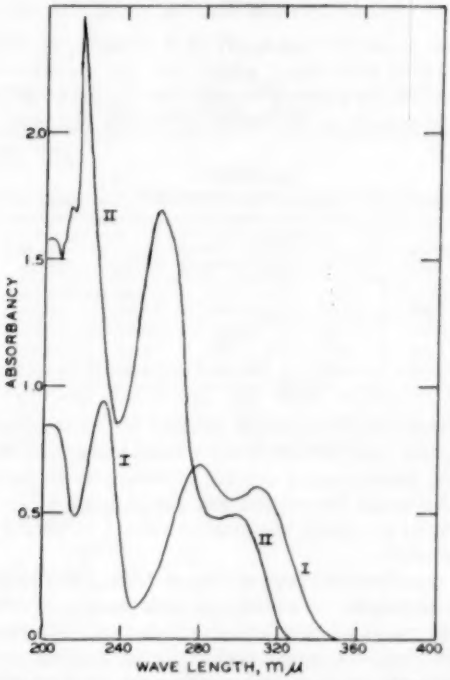

Figure 2. Absorption curves for vanillin and vanitrope in acidic solution (pH = 4.6). I 20 mg. vanillin per liter. II 20 mg. vanitrope per liter.

However, should the ratio be large, a slight error will occur. At λ 256 mμ, vanitrope has a maximum absorption while that of vanillin is near a minimum and the difference in absorbance is large. Since conformance to Beer's Law is characteristic of both constituents in the mixture, an analysis for each of the two may be made by observing the absorbancies at both 347 and 256 mμ and calculation of each from a solution of two equations involving the known absorbancies per unit weight. Table 3 gives the results of the analysis of several mixtures in which the proportions of the constituents were varied.

The process of calculation is here indicated. If X and Y are respectively the centigrams of vanillin and vanitrope present per liter in a mixture of the two components, and from Table 2 the appropriate absorbancy values for each are employed as coefficients, the following simultaneous equations may be set up.

(a) $0.42X + 0.815Y =$ observed A at 256 mμ.
(b) $1.67X + 0.02Y =$ observed A at 347 mμ.

Whence, by solution of the equations above

$$Y = \frac{3.98 \times \text{A at } 256\ m\mu - \text{A at } 347\ m\mu}{3.22}$$

$$X = \frac{\text{A at } 347\ m\mu - 0.024}{1.67}$$

As has been mentioned previously, when Y is small this term can be neglected in the calculation of X.

DISCUSSION

If the solution is not held at a pH of 9 or more, vanillin will not have attained its maximum absorbancy at 347 mμ. On the other hand, if the pH is increased above 10, the absorption peak for vanitrope at 256 mμ decreases markedly and the nature of the curve is greatly changed. Therefore, pH control is very essential. It should be stressed too, that since vanillin absorption changes rapidly with change in wave length near 256 mμ, an instrument of good resolving power and a careful checking as to wave length setting is imperative. Although the results are not of high precision, particularly when the quantities are small, the method should be useful for examination of flavoring materials.

TABLE 3

Analysis of vanillin-vanitrope mixtures from ultraviolet absorbancy data for basic solution

Sample	pH	Absorbancy		Vanillin		Vanitrope	
		at λ 347 mμ	at λ 256 mμ	Present	Found	Present	Found
				mg. per liter		*mg. per liter*	
III	8.99	1.68	2.06	10.00	9.82	20.00	20.24
IV	9.04	1.66	0.81	10.00	9.88	5.00	4.85
VII	8.98	0.44	1.72	2.50	2.40	20.00	19.90
VIII	9.05	0.41	0.50	2.50	2.40	5.00	4.90

Analysis of vanillin-vanitrope mixtures from absorbancy values in both acid and basic solutions. Because vanillin barely attains its maximum absorption at 347 mμ at a basicity near which vanitrope undergoes a change in absorption characteristics, the possibility of making one set of observations in a more basic solution and another set in acid solution gave some promise of improvement in the method. This change in detail can easily be effected by use of aliquots of the same solution, adding the necessary reagents and putting through parallel dilutions.

Reference to Figure 2 indicates that wave length 256 mμ is again important as a point of evaluation in the acid range since the two constituents still have a maximum difference in absorbancy near this wave length. From the standpoint of pH control it is more favorable in that the pH may vary from 6 to less than 4 without affecting the results.

Another wave length of interest for the acid mixture is located at 295 mμ. Here, there is a secondary maximum for vanitrope and a minimum for vanillin. Since both curves are tending to be flat in this region, the setting for the wave length is not so critical. However, differences in absorbancy are much smaller than at 256 mμ. To attain the pH for the acid condition, 10 ml. of monosodium phosphate is added per 100 ml. of final solution, instead of the disodium phosphate.

For the evaluation of the absorption at 347 mμ in the basic solution, the procedure is the same as that used in the previous experiments except that 0.5 g. of sodium carbonate was added along with the disodium phosphate. A pH of 10.7 was observed for these samples.

Mixtures of vanitrope and vanillin were prepared and absorbancies measured at the specified wave lengths. The nature of these mixtures and the results are given in Table 4. The principle in the calculations is

identical to that already described and is based on data from Table 2 and Table 4.

TABLE 4

Analysis of vanillin-vanitrope mixtures from ultraviolet absorbancy data for basic and acidic solutions

	Absorbancy			Vanillin			Vanitrope		
	a	b	c						
Sample	λ 347 mμ at pH = 10.7	λ 295 pH = 4.5	λ 256	Present	Found (a) (b)	(a) (c)	Present	Found (a) (b)	(a) (c)
1	1.72	0.95	1.56	10.00	10.10	10.10	15.00	15.8	15.6
2	1.69	0.673	0.63	10.00	10.00	10.07	5.00	5.15	4.92
3	0.91	0.79	1.86	5.00	5.20	5.20	20.00	20.50	20.30
4	0.84	0.40	0.54	5.00	4.97	5.05	5.00	5.05	4.96

For the calculations from absorbancies at 256 mμ in acid solution and 347 mμ in basic solution the initial and final equations are as follows:

(a) $0.21X + 0.865Y = A$ at 256 mμ (acid solution)

(b) $1.67X + 0.02Y = A$ at 347 mμ (basic solution)

whence

$$Y = \frac{7.95 \text{ A at } 256 \text{ m}\mu - \text{A at } 347 \text{ m}\mu}{6.85}$$

$$X = \frac{\text{A at } 347 \text{ m}\mu - 0.02Y}{1.67}$$

For the calculations from absorbancies at 295 mμ in acid solution and 347 mμ in basic solution the initial equations are:

$0.555X + 0.245Y = A$ at 295 mμ (acid solution)

$1.67X + 0.02Y = A$ at 347 mμ (basic solution)

whence

$$Y = \frac{3.01 \times \text{A at } 256 \text{ m}\mu - \text{A at } 347 \text{ m}\mu}{7.18}$$

$$X = \frac{\text{A at } 347 \text{ m}\mu - 0.024}{1.67}$$

DISCUSSION

The results in Table 4 are similar in nature to those of Table 3. Although no definite improvement in precision is evident, the maximum difference in amount found and amount present is of the order of 5% and most values are near 1%. These results are equal to or better than those obtained by previous methods for vanillin in mixtures with other flavoring agents. The procedures are simple and rapid. The selection of the conditions which will find use in actual practice may depend upon extraneous materials present in the samples or the reagents employed in a preliminary treatment. Further experiments will be devoted to an examination of commercial products.

SUMMARY

The spectrochemical characteristics of propenyl guaethol and vanillin have been investigated relative to the development of procedures for analysis of products containing the two compounds.

Consideration of the colorimetric method employing the Folin-Denis reagent has shown that propenyl guaethol reacts to give even greater color intensity than vanillin itself. This indicates that the method is suitable for the determination of either substance when present alone. It can be applicable to the analysis of a mixture of the two compounds if one constituent is determined directly by another method.

A study of the ultraviolet absorption spectra of each compound has been made under both acidic and basic conditions. Propenyl guaethol has an absorption maximum at λ 256 mμ where an equal concentration of vanillin shows lesser absorption. In alkaline solution it has almost negligible absorption at λ 347 mμ where vanillin has a maximum. A procedure has been formulated for the analysis of vanitrope-vanillin mixtures based on the absorbancy of solutions of pH 9 at these wave lengths.

Alternate procedures employing other conditions of pH of the solutions and absorbancy at other wave lengths are presented.

Acknowledgments

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CROP, LIGHT INTENSITY, SOIL pH, AND MINOR ELEMENT EFFECTS ON THE YIELD AND VITAMIN CONTENT OF TURNIP GREENS

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It is well recognized that factors affecting the vitamin content of plants usually cause composition changes of considerable nutritional importance. Most previous investigations of these effects have considered a single factor rather than studying several factors simultaneously.

Harmer and Sherman *(9)* and Hester *(10)* have indicated that low ascorbic acid content of certain plants is associated with a deficiency or low availability of manganese in the soil. In these instances, applications of manganese increased the ascorbic acid content of plants. Gum, Brown, and Burrell *(4)*, Lyon and Beeson *(11, 12)*, and Lyon, Beeson, and Ellis *(13)*, report either no effect, or slight but inconclusive effects, on the ascorbic acid content of certain plants when minor element application was made in amounts ranging from deficient to toxic concentrations.

Probably the largest and most consistent increases in the ascorbic acid content of plants have been caused by increases in light intensity, a phenomenon reported by Aberg *(1)*, Hammer, Lyon, and Hamner *(6)*, Bernstein *(2)*, Hamner and Parks *(8)*, and Somers, Kelly, and Hamner *(21)*. The influence of fertilizers on the ascorbic acid content of turnip greens has been studied by workers of the Southern Cooperative Group at several locations in the South. Reports on these studies have been made by Reder, Ascham, and Eheart *(14)*, Reder *et al. (15)*, and Sheets *et al. (18)*. Although the fertilizer treatments produced no consistent effects, increases in the ascorbic acid content of the greens occurred at locations having high light intensities prior to harvest.

Work by Bernstein, Hamner, and Parks *(2)* and Hamner and Maynard *(7)* showed that increases in the carotene content of turnip greens and tomatoes respectively were obtained with increased light intensity. Gustaveson *(5)* found that increasing light intensity caused proportional increases in the thiamine content of tomatoes, potatoes, beans, peas, and New Zealand spinach; and consistent but less pronounced increases in the riboflavin content of these vegetables. Excellent literature reviews on the effect of environment on the chemical composition of plants have been prepared by Somers and Beeson *(20)* and Richardson *et al. (16)*.

Recognizing the limitations of single-factor experiments for obtaining information of this type, an experiment was initiated in 1950 at Blacksburg, Virginia, in which the effect of crops, soil pH, light intensity, and minor elements, and their interactions, on the nutritive value and yield of turnip greens, were studied simultaneously.

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MATERIALS AND METHODS

Spring and fall plantings of the Seven Top variety of turnips were made in 1950, 1951, and 1952 at Blacksburg, Virginia. Because of failures of three of these crops, results for only the 1950 spring and fall crops and the 1952 fall crop are discussed in this paper. The greens were grown on permanent plots on Groseclose silt loam type of soil, were planted in rows 2 feet apart, and thinned to 4 inches in the row. Approximately one-fourth of an acre was required for the study. The entire experimental area was fertilized annually with a uniform application of 5-10-5 fertilizer, broadcast and disked into the soil.

A split, split, split, factorial design was used which included 3 crops, two levels each of soil pH and light intensity, and 3 minor element treatments. The minor element treatments were replicated 6 times and were randomized within each replication. The same position was used for each plot for each of the 3 crops. (Three crops $\times$ 2 pH levels $\times$ 2 light intensities $\times$ 3 minor element treatments $\times$ 6 reps. = 216.)

The 2 levels of soil pH were designated as: (a) low pH (4.8 to 5.1), and (b) high pH (6.6 to 6.9). Lime was applied to the high pH plots in sufficient amounts to maintain a pH of 6.6 to 6.9. No lime was applied to the low pH plots. It should be pointed out that, in addition to increasing the pH of the soil by use of lime applications, it is possible that plant composition and yield would be influenced to some extent by the added calcium from the lime.

Two light intensity levels were produced by shading one-half of each limed and one-half of each unlimed plot with tobacco cloth (thread count 32×28). The cloth was suspended 2 feet above the ground on heavy wires, which in turn were supported by wooden stakes. A Weston light meter indicated that the tobacco cloth reduced the light intensity approximately 50% on a bright day. Shading was started 10 days prior to harvest.

Within each light intensity level, three randomized minor element treatments were made as follows: (a) no minor elements, (b) minor elements applied as a foliar spray, and (c) minor elements applied to the soil. (For convenience, these treatments will be referred to hereafter as *no minor elements*, *spray minor elements*, and *soil minor elements.)* A commercial preparation known as ES-MIN-EL[b] was used as a source of the minor elements. It was applied as a foliar spray, 2 weeks before harvest, at the rate of 15 pounds per acre (mixed with 7.5 pounds of lime and dissolved in 375 gallons of water); and to the soil as a side dressing at the rate of 50 pounds per acre after the plants had been thinned.

Each minor element plot consisted of four 16-foot rows two feet apart. Leaf samples for analysis and complete plant harvest for yield data were taken from the center 10 feet of the two middle rows when the greens reached commercial maturity. One leaf of medium age was taken from each plant in the plot area sampled. The samples were taken successively by replication on the complete experiment between 8:00 and 10:00 a.m. in a single day, placed in paper bags, brought to the laboratory, and kept under refrigeration until analyzed.

Ascorbic acid analyses and preparation of the samples for carotene analyses by boiling them in ethanol were done concurrently and were usually completed in three hours or less after harvest. Preparation of the samples for thiamine and riboflavin analyses by: (a) grinding in 0.04% sulfuric acid, and (b) autoclaving was usually completed in 5 and 7 hours or less after harvest, respectively. These procedures insured minimum losses of the constituents during preparation of the samples for analysis.

Dry matter and ascorbic acid determinations were made and yield data were taken on all samples from the three crops and, in addition, carotene, thiamine, and riboflavin analyses were made on the 1952 fall samples. The dry matter content was determined

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[b] ES-MIN-EL was supplied by the Tennessee Corporation, Atlanta, Georgia, and had the following guaranteed analysis: 9.87% Mn., 3.81% Cu., 3.50% Zn., 3.00% Fe., 2.42% Mg., 0.95% Ca., 0.16% B., and a trace of Cl. The first six elements were supplied as the sulfates and B. as borax. No inert material was reported as being present. The authors wish to express their appreciation to the Tennessee Corporation for supplying this material.

by heating the fresh unground greens in a forced draft oven at 70°C. ± 2° for the first 2 hours and at 95°C. ± 2° for 46 hours. The reduced form of ascorbic acid (Heinz-Kanapaux Method), carotene, thiamine, and riboflavin were analyzed by methods used by the Southern Cooperative Group *(17)*. The Analysis of Variance Method, described by Snedecor *(19)*, was used for statistical analysis. Duncan's Multiple Range Method *(3)* was used for testing the significance of the data. Only data which proved to be statistically significant are given. In order that composition results for ascorbic acid, carotene, thiamine, and riboflavin may be compared on the same basis, all data for these constituents are presented on the dry basis.

TABLE 1

Factors affecting the dry matter, yield, and ascorbic acid content (dry basis) of turnip greens

Factors	Spring 1950 crop	Fall 1950 crop	Fall 1952 crop	Mean
		Yield—T/A		
High soil pH	6.03	7.29	4.19	5.83[a1]
Low soil pH	5.85	5.42	3.95	5.08[b]
No minor elements applied	5.75	6.31	4.51	5.52[a b]
Minor elements applied as a foliar spray	6.01	6.49	3.06	5.19[b]
Minor elements applied to the soil	6.06	6.26	4.63	5.65[a]
Mean	5.94[b]	6.35[a]	4.07[c]	5.45
		Ascorbic acid—mg./g.		
Mean	12.30[a]	11.07[c]	11.36[b]	11.58[5]
		Dry matter—%		
100% light intensity	11.17	11.34	9.55	10.68[a]
50% light intensity	9.99	10.66	9.79	10.15[b]
Mean	10.58[b]	11.00[a]	9.67[c]	10.42[4]

Factors	%[2] range	F[3] value	% C.V.	No. samples/ mean
		Yield		
pH	14.8	15.1*	26.2	108
Minor elements (M.E.)	8.9	4.0*	30.6	72
Crops (C)	56.0	128.7**	18.4	72
pH × C	84.5	20.1**		36
M.E. × C	112.1	9.2**		24
		Ascorbic acid		
Crops	11.1	54.4**	6.3	72
		Dry matter		
Crops (C)	13.7	144.9**	3.8	72
Light intensity (L)	5.2	23.3**	4.6	108
C × L	18.7	40.9**		36

[1] Data having superscript "a" are significantly different from those not having "a"; those having "b" from those not having "b," etc. (3).

[2] [(Maximum value/minimum value) 100]—100. Example (crop yield): [6.35/4.07) 100] —100 = 56.0% range.

[3] * and **—Significant at 5 and 1% level, respectively.

[4] Lbs. D.M./A. = Yield × 2000 × respective % D. M. Example: 5.45 × 2000 × 10.42% = 1136.

[5] Mg. ascorbic acid/A. = Lbs. D.M./A. × respective mg.a.a./g. × 453.6. Example: 1136 × 11.58 × 453.6 = 59,671.

RESULTS AND DISCUSSION

A range[c] of 14.8% shows that there was a larger yield from the high than from the low pH plots. The greens from the *soil minor element* plots were approximately 9% higher in yield than those from the *spray minor element* plots; there was no yield difference between the *no minor element* greens and those from the *soil* or *spray minor element* treatments. The 56% range shows that crop had 3.8 and 6.3 times[d] more influence on yield than did pH and minor elements. The minor element × crop interaction indicated 33% more variation in yield than was shown by the pH × crop interaction; respective ranges being 112.1 and 84.2%. In the high pH treatment there was an increase in yield of the 1950 fall over the 1950 spring crop; the reverse was true for the low pH treatment. The 1952 fall crop yield was much lower from both the low and high pH plots than from the corresponding 1950 spring and fall crops. The increase in yield of the 1950 fall over the 1950 spring crop was about 2.5 times as much for the *no minor element* and the *spray minor element* treatments as for the *soil minor element* treatment. The low 1952 fall yields for the three minor element treatments (particularly for the foliar spray) also contributed materially to the highly significant minor element × crop interaction.

The ascorbic acid content of the greens was influenced only by crops; significant differences between each of the three crops resulting. The 11.1% range showed a substantial difference in ascorbic acid content as a result of crop effect. The fact unaccounted for—that differences in light intensity had no significant effect on the ascorbic acid content of the greens—was contrary to results obtained by numerous investigators *(1, 2, 6, 8, 14, 15, 21)*, all of whom reported increases in the ascorbic acid content of plants with increased light intensity.

Both crop and light intensity influenced the dry matter content of the greens, but the crop effect was approximately 2.5 times that of light intensity, as indicated by ranges of 13.7 and 5.2%, respectively. Greens from the 100% light intensity 1950 fall crop showed a slight increase in dry matter content over those of the 1950 spring crop, and a considerably greater increase over the 50% light intensity greens. There was an increase in the percentage of dry matter of the 100% over that of the 50% light intensity greens in both the 1950 spring and fall crops; the reverse was true for the greens from the 1952 fall crop. The dry matter content of the 1952 fall greens from both light intensities was lower than simliarly treated greens from the 1950 spring and fall crops.

Per acre yields of dry matter and ascorbic acid can be calculated by use of formulas in footnotes 4 and 5, Table 1.

Carotene content (Table 2) was affected only by light intensity, as shown by a 9.1% increase of the 50% over the 100% light intensity greens. This was the reverse of results obtained by Bernstein, Hamner, and Parks *(2)*.

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[c] See footnote 2, Table 1.

[d] This, and subsequent comparisons of factor effects, has been made by comparing the percentage range (footnote 2, Table 1) of a given constituent for a particular factor with the percentage range of the same constituent for the other factor under consideration.

TABLE 2

Factors affecting the carotene, thiamine, and riboflavin contents (dry basis) of turnip greens (1952 fall crop)

Factors	Carotene γ/g.	Thiamine—γ/g.		
		Low pH	High pH	Mean
100% light intensity	635.02[b1]	15.19	14.17	14.68[a]
50% light intensity	692.58[a]	14.05	14.17	14.11[a]
Mean	665.30	14.62[a]	14.17[a]	14.40
	Thiamine γ/g.	Riboflavin—γ/g.		
		Light intensity		
		100%	50%	Mean
No minor elements applied	14.24[b]	37.76	37.02	37.39[a]
Minor elements applied as a foliar spray	14.05[b]	37.47	37.76	37.61[a]
Minor elements applied to the soil	14.90[a]	38.57	36.15	37.36[a]
Mean	14.40	37.93[a]	36.98[b]	37.45

Factors	%[2] range	F[3] value	% C.V.	No. samples/ mean
		Carotene		
Light intensity (L)	9.1	4.7**	5.3	36
		Thiamine		
Minor elements (M.E.)	6.0	5.3**	7.5	24
L × pH	8.1	5.1*		18
		Riboflavin		
Light intensity (L)	2.6	9.5*	3.5	36
L × M.E.	6.7	3.5*		12

[1] See footnote 1, Table 1.
[2] See footnote 2, Table 2.
[3] * and ** = Significant at 5 and 1%, respectively.

The thiamine content of the greens from the *soil minor element* plots was significantly greater than those from the *spray* and the *no minor element* plots while there was no difference between those from the latter two treatments. The comparatively high thiamine content of the greens from the low pH-100% light intensity plots (while the other three values were practically the same), accounted, in a large measure, for the significant pH × light intensity interaction.

The 2.6% range in riboflavin content between the 100% and 50% light intensity greens, though significant, represented a difference which was too small to be important. The significant light intensity × minor element interaction can be accounted for largely by the comparatively large difference in riboflavin content of the 50% and 100% light intensity greens from the *soil minor element* plots while the differences among the other four values were practically negligible.

Attention should be called to the fact that the coefficients of variation, expressed as per cent (% C.V.) were, with the exception of yield data, well below the accepted figure for the various constituents studied, under the field conditions for this type of experiment.

In Table 3, composition means obtained in this study have been compared with data for the same constituents given in USDA Handbook 8 *(22)*.

TABLE 3

Turnip green mean composition (dry basis) values compared with U.S.D.A. Handbook 8 (22) values

Constituent	This study:		U.S.D.A. Handbook 8 values	% difference from U.S.D.A. values
	No. samples/mean	Means		
Dry matter %	216	10.42	10.50	− 3.5
Ascorbic acid mg./g.	216	11.58	12.95	−10.6
Carotene γ/g.	72	665.30	545.14	+22.0
Thiamine γ/g.	72	14.40	8.57	+68.0
Riboflavin γ/g.	72	37.45	43.81	−14.5

The –3.5% difference in dry matter content was of little importance. The ascorbic acid and riboflavin content was considerably lower than USDA values, as indicated by differences of –10.6 and –14.5%, respectively. The carotene content was 22.0% higher, and there was a large difference of +68.0% in the thiamine content of the greens.

SUMMARY AND CONCLUSIONS

In a study of the effects of crops, light intensity, soil pH, and minor elements, on the yield, and ascorbic acid and dry matter contents of three crops, and on the carotene, thiamine, and riboflavin contents of one crop of turnip greens, the following pertinent results were obtained:

Yield was highest in the 1950 fall crop and lowest in the 1952 fall crop; highest in the high-pH plots, and in the plots to which minor elements were applied to the soil. Greater variability in yield was indicated by the minor element × crop than by the pH × crop interaction.

Ascorbic acid content was highest in the 1950 spring crop and lowest in the 1950 fall crop.

Dry matter content was highest in the 1950 fall crop and lowest in the 1952 fall crop and an increase was obtained with high light intensity. Variability in dry matter content was indicated by the significant crop × light intensity interaction.

Carotene content decreased with high light inetnsity.

Thiamine content increased slightly when minor elements were applied to the soil and considerably with a combination of high light intensity and low soil pH.

Riboflavin content increased to a small degree with high light intensity and more by a combination of high light intensity and minor elements applied to the soil.

Mean values obtained in this study were considerably lower for ascorbic acid and riboflavin contents, higher for carotene content, and much higher for thiamine content than corresponding values given in USDA Handbook 8 *(22)*.

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CHRONIC TOXICITY STUDY OF PHYTIC-ACID–TREATED SOYBEAN OIL FOR RATS

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Phytic acid (the hexaphosphoric acid ester of inositol) is a natural constituent of cereal grains and oilseeds. It has been shown to be highly effective in counteracting the adverse effect of metallic contamination in edible oils. When added to regularly processed oils at the 0.01% level, phytic acid brings about a marked improvement in the organoleptic and oxidative stability of these oils *(3)*.

Because it is widely distributed in nature (usually as phytin, the calcium and magnesium salt), this acid is frequently ingested in large amounts by man. A recent bibliography on phytin *(4)* confirms the fact that a large amount of work has been published dealing with the biochemical and nutritional aspects of phytic acid, including its anticalcifying action in the animal body.

Phytic acid is available as a by-product from corn steep liquor. If it were used to improve the quality of edible oils, it would be ingested by man, and a health hazard might be involved. This work was undertaken to determine whether any toxicological manifestations would be encountered in rats ingesting phytic-acid-treated soybean oil for prolonged periods of time.

EXPERIMENTAL PROCEDURE

Generally speaking, it is desirable in a study of chronic toxicity to use sufficiently high levels of the substance in question to make sure that any toxic effects that develop are clearly revealed. Then, specific effects with lower dosage levels are more easily detected. With this purpose in mind, phytic acid *per se* was given to several groups of rats at the same time the soybean oils were being tested.

Because of the known relationship of phytic acid and calcium, two basic diets were used (Table 1), one (Purina Laboratory Chow) containing nearly twice as much calcium as the other (Addis). Also 0.7% calcium was added to the Addis diet in order to increase the calcium content to a level comparable to that of the Purina ration

TABLE 1

Comparative proximate analysis of diets

Dietary constituent	Purina Lab. Chow[1]	Addis
Protein (N × 6.25)	23.8%	17.0%
Moisture	9.8	11.4
Crude fat (ether extract)	6.0	4.5
Crude fiber	4.6	1.7
Ash	6.4	3.2
Nitrogen-free extract (by diff.)	49.4	62.3
Calcium	1.17	0.65
Phosphorus	0.87	0.50

[1] Use of this commercial feedstuff does not imply indorsement by this agency.

(1.17%). The Addis diet was prepared in our own laboratory as needed and had the following composition: degerminated yellow cornmeal 73%, crude casein 10%, linseed oil cake meal 10%, ground alfalfa 2%, bone ash 1.5%, sodium chloride 0.5%, and cod liver oil U.S.P. 3%. The composition of the Purina diet, consisting of numerous natural products, is not known percentage-wise.

The odorless and flavorless characteristics of vegetable oils destined for human consumption are achieved only through the usual commercial practices of refining, bleaching, and deodorizing. During the steam-deodorization processing of soybean oil *(3)* the phytic acid is added. Aqueous solutions of phytic acid (approx. 30%) from two commercial sources (Corn Products and A. E. Staley Mfg. Co.) were used by the Northern Utilization Research Branch in the preparation of the phytic-acid–treated soybean oil used in these experiments. Ten times the usual amount of phytic acid (0.1%) was used during the deodorization process in order to subject the rats to a level of intake many times in excess of any calculated human intake. This oil and untreated oil were fed at a single high level of 10% (substituted at the expense of the entire diet). The average amount of vegetable oils ingested by humans is roughly 0.6% of the total diet, based on figures taken from U.S.D.A. Misc. Publication No. 452 dated 1941.

Male and female weanling albino rats from our stock colony were used throughout. Five rats per group were caged together in solid-bottom cages with wood shavings as litter. Free access to the respective diets and water was permitted at all times. Food consumption for each group and the body weight of each rat were recorded at weekly intervals during the first 200 days. Half of the animals were sacrificed at approximately 200 days and the remainder at somewhat over 400 days. At the time of sacrifice gross post mortem examinations were made and organs weighed. Histopathological examinations of vital organs were made routinely on all animals. Hemoglobin concentrations and length and weight of femurs were determined on a few rats receiving 2% phytic acid *per se*.

RESULTS

Gains in weight, food consumption, and mortality for all rats on the various diets used are shown in Table 2. Without exception, the weight gains for both sexes were greater when 10% phytic-acid–treated or untreated soybean oil was added to the Addis diet. However, when the phytic acid solutions were added to the Addis diet at levels equivalent to 2% phytic acid, definite growth inhibition resulted. The addition of calcium as calcium carbonate failed to counteract the growth inhibition. When the same amount of phytic acid was added to a Purina diet no growth inhibition was observed.

Food consumption was altered very little if any by the various dietary supplements. Likewise, there was no evidence of an increased mortality due to phytic acid during the 400-day experiment. No gross anatomical changes were observed in any of the organs of rats subjected to diets containing 10% treated or untreated soybean oil. The testes of 400-day-old rats receiving 2% phytic acid added to the Addis diet were found to be appreciably smaller than those of the controls.

Hematoxylin eosin stained sections of the following organs were prepared: heart, liver, lungs, thyroid, trachea, kidney, bladder, adrenals, gut, hypophysis, ovaries, testes, spleen, and pancreas. The histopathological examinations were made by Dr. L. J. Rather of the Stanford Medical School, San Francisco, California. In addition to confirmation of the moderately severe testicular atrophy of rats receiving 2% phytic acid added to the Addis diet, it was found that 13 out of 18 rats (both sexes) receiving 10% soybean oil (treated or untreated) had vacuoles in the liver cells; whereas 2 of 9 control rats had such vacuoles. Fat-stained sections of livers confirmed the presence of fat to account for the vacuoles of the liver cells.

Hemoglobin concentrations and bone measurements of animals receiving 2% phytic acid *per se* did not differ from control values.

DISCUSSION

The failure to observe any definite evidence of toxicity from phytic-acid–treated soybean oil fed to rats for more than 400 days is not surprising

TABLE 2

Effect of phytic acid and soybean oil on rat mortality, body weight, and food consumption

Dietary supplement	Number of rats				Body weight gain[1]—grams		Food intake
	Sex	At start	202 days	423 days[2]	202 day gain	423 day gain[2]	Gm/rat/day
Control (Addis)	M	5	3	..	327.4 ± 5.0	.....	13.7
Control (Addis)	M	5	5	5	296.0 ± 10.7	368.6 ± 12.5	12.3
+ 10% untreated SBO[3]	M	5	5	..	327.2 ± 13.7	.....	11.3
+ 10% untreated SBO	M	5	5	5	314.6 ± 7.4	372.8 ± 7.5	11.2
+ 10% PA[4] treated SBO	M	5	5	..	344.8 ± 14.5	.....	12.1
+ 10% PA treated SBO	M	5	4	4	351.2 ± 7.4	397.4 ± 3.6	12.3
+ 2% PA (Staley)	M	5	5	..	252.4 ± 14.4	.....	12.7
+ 2% PA (Staley) + 10% untreated SBO	M	5	5	..	269.2 ± 10.0	.....	11.0
Control (Purina)	M	5	5	..	244.8 ± 15.4	.....	13.4
Control (Purina)	M	5	5	3	261.8 ± 11.1	290.5 ± 8.6	14.1
+ 2% PA (CP)[5]	M	5	4	..	269.3 ± 4.2	.....	14.9
+ 2% PA (CP)	M	5	5	4	261.8 ± 14.5	308.3 ± 26.2	15.2
Control (Addis)	M	5	5	..	301.6 ± 9.8	.....	13.5
Control (Addis)	M	5	5	5	312.6 ± 15.5	353.2 ± 20.0	13.5
+ 1% PA (CP)	M	5	4	..	271.8 ± 10.8	.....	13.1
+ 1% PA (CP)	M	5	5	5	258.5 ± 14.1	323.3 ± 15.5	12.9
+ 2% PA (CP) + 0.7% calcium	M	5	4	..	223.5 ± 8.8	.....	13.5
+ 2% PA (CP) + 0.7% calcium	M	5	5	5	250.4 ± 12.1	305.0 ± 18.4	13.7
Control (Addis)	F	5	5	..	206.4 ± 16.2	.....	9.7
Control (Addis)	F	5	5	5	202.4 ± 7.6	262.4 ± 15.5	9.9
+ 10% untreated SBO	F	5	5	..	207.8 ± 10.7	.....	9.3
+ 10% untreated SBO	F	5	5	4	232.0 ± 8.8	282.4 ± 7.2	9.4
+ 10% PA treated SBO	F	5	4	..	228.4 ± 16.3	.....	10.5
+ 10% PA treated SBO	F	5	5	4	233.4 ± 15.6	295.5 ± 16.8	9.0
+ 2% PA (CP)	F	5	5	..	155.4 ± 11.1	.....	9.9
+ 2% PA (CP) + 10% untreated SBO	F	5	5	..	199.0 ± 5.7	.....	11.9

[1] Standard error of the mean, $\sqrt{\frac{Ed^2}{n(n-1)}}$

[2] A dash (—) indicates animals were sacrificed at 202 days.
[3] SBO = Soybean oil, [4] PA = phytic acid; [5] CP = Corn Products Company.

if due consideration is given to the fact that the phytic acid added to the oil cannot be detected chemically after the deodorization process is completed. The insolubility of phytic acid in oil probably accounts for the fact that over half of the added phytic acid can be recovered as a water-soluble residue remaining in the deodorization flasks *(3)*.

The marked growth inhibition resulting when 2% phytic acid was added to one basal diet (Addis) with added calcium is in striking contrast to the complete protection afforded by the Purina diet when 2% phytic acid was added. These observations coincide with the findings of Wilson and DeEds on the importance of diet in studies of chronic toxicity *(6)*. An explanation as to why the Purina diet (which is inferior to the Addis diet for supporting an optimum rate of growth) is able to completely protect rats from growth inhibition and testicular damage due to phytic acid is not readily apparent. Certainly, here is an additional example of the importance of the basal diet one selects for use in attempting to evaluate the toxicity of a substance when ingested for prolonged periods of time by rats. In the case of the Purina diet plus added phytic acid it might be erroneously concluded that this particular acid is non-toxic, yet definite toxic effects were observable when the same amount of phytic acid was added to the Addis diet, which by itself is nutritionally superior, as judged by rate of growth.

The mechanism by which phytic acid exerts its beneficial effects on soybean oil may be by chelation with iron and copper, since it was shown that iron and copper added to soybean oil could be inactivated by phytic acid *(3)*. In rats receiving high levels of phytic acid, would its sequestering action produce an anemia? The failure to observe lowered hemoglobin values is not surprising inasmuch as recognized chelating agents such as 8-hydroxy quinoline *(1)*, dithio-oxamide *(5)*, and quercetin *(2)* also have been found incapable of causing anemia in rats ingesting high levels of these substances in the diet for long periods of time.

Failure to reverse the toxic effects of phytic acid *per se* with calcium suggests that other factors are involved in addition to a simple calcium complex being formed.

SUMMARY

Rats receiving 10% phytic-acid–treated soybean oil in the diet for more than 400 days showed no evidence of toxicity as judged by growth rates and gross and histopathological examination of the tissues after autopsy. These negative findings plus the failure to chemically demonstrate the presence of the added phytic acid in the oil after the deodorization process indicate that phytic-acid–treated soybean oil is non-toxic.

Toxic symptoms including decreased rates of growth and testicular atrophy were evident when 2% phytic acid *per se*, with or without added calcium, was ingested with a basal diet consisting primarily of corn meal, linseed meal, and crude casein. These toxic symptoms did not develop when the same amount of phytic acid was added to a commercial rat diet, and the importance of diet in chronic toxicity tests is again reiterated.

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STUDIES ON ENZYMATIC TENDERIZATION OF MEAT. I. BASIC TECHNIQUE AND HISTOLOGICAL OBSERVATIONS OF ENZYMATIC ACTION[a, b]

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Recent years have witnessed a rapidly increasing interest in the use of proteolytic enzymes of plant origin for various industrial purposes. One such enzyme, known for centuries, is papain, which is present in large quantities in the juice of the fruit of the tropical melon tree, *Carica papaya*. There is considerable information available concerning this enzyme *(4, 7)*, most of which relates to chemical methods of assay and factors influencing enzyme activity *(6)*. Among other uses, it has become the principal agent in many of the meat tenderizers available on the market today. Other enzyme preparations are being introduced; for example, a proteolytic enzyme derived from the pineapple plant and known as "bromelin" has been suggested *(1)* and its meat tenderizing potentiality was implied *(2)*. Most recently, proteolytic enzyme preparations derived from other sources such as bacteria and fungi have been produced to broaden the scope of industrial enzyme usage. One such enzyme preparation,[c] bearing the trade name Rhozyme P-11, is a potent proteolytic enzyme of fungal origin *(5)*.

It is becoming increasingly apparent that any proteolytic enzyme can be a potential meat tenderizer. The enzymes mentioned above have been chemically assayed as to their specific potency and other properties. The most commonly used methods for measuring their activity are: gelatin-digestion, milk-clotting, hemoglobin-splitting, ground beef-digestion, and the like; but so far as we are aware, nothing is known of the effect of these enzymes on the structural characteristics of meat. Yet, conceivably, the tenderizing property of any proteolytic enzyme must depend on its ability to break down, degrade, or hydrolyze definitive structural (protein) components of muscle tissue. Since present histological techniques are available for disclosing the manner in which structural changes may take place as a direct result of the enzymatic action, it would appear desirable to follow the effect of various enzyme preparations on meat by histological examination. The "histological criteria" of enzymatic action thus sought are largely of a qualitative nature; nevertheless, it is not entirely impossible that by following a sequence of structural alterations or/and by correlating the order of events with the enzymatic activity levels involved, one may hope to be able to quantitate the enzymatic action on the basis of structure.

In view of these considerations we decided to undertake the present

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[a] This paper was presented before the Sixty-eighth Annual Meeting of the American Association of Anatomists, Philadelphia, Pennsylvania, April 8, 1955 *(9)*.

[b] Journal Paper No. 118, American Meat Institute Foundation.

[c] Rohm and Haas Co.

studies with the following broad objectives in mind: (a) to define histologically the nature of enzymatic action on the various constituents and structural characteristics of muscle tissue; (b) to determine the relationship, if any, between the structural changes brought about directly by the enzymatic action and the resulting effect on the organoleptic properties of the meat, especially tenderness; (c) to develop and formulate, ultimately, practical means of producing effective enzymatic tenderization of meat for purposes of improving the acceptability of low grade meat cuts and other meat products.

Our objective in this paper was confined to (a) the development of a basic technique of enzyme application to muscle tissue—one that insures speedy penetration of the enzyme into the substrate as evenly and uniformly as possible; (b) a comparison of this technique with two conventional methods of enzyme application for their relative efficiency; and (c) tracing of the action of two proteolytic enzymes of widely different origins on the various components of meat.

MATERIALS AND METHODS

Three kinds of muscle tissues were used for enzyme treatment. They consisted of frozen-dried, ½-inch thick pork chops *(longissimus dorsi)* and beef ribeye *(longissimus dorsi)* steaks[d] and raw beef outside round, the *semitendinosus* muscle. The chops and steaks contained approximately 3% residual moisture and were capable of rehydrating back to 70% or higher water content in 5 to 15 minutes *(8)*.

Two proteolytic enzymes were tested in the present series of experiments. One was a papain preparation[e] obtained from the market, which also contains salt and dextrose. The other enzyme preparation[f] contains a proteolytic enzyme of fungal origin. This enzyme was available in two preparations: one, a product blended with salt and dextrose, and the other, a concentrate stated to be approximately 3½ times as potent as the former. They will be referred to respectively as Rhozyme P-11 and Rhozyme concentrated.

Application of the enzymes was carried out by three different methods. In the case of raw beef *semitendinosus* tissue, blocks measuring approximately 15 × 20 × 5 mm. with muscle fibers running either parallel or transversely to the sample's broad surfaces were immersed in an aqueous solution of the enzyme, or the enzyme preparation, in powder form, was spread over the sample for a specified length of time.

For the frozen-dried tissues, a new technique was developed to achieve a maximum degree of tissue-enzyme contact. Due to the spongy nature of frozen-dried muscle tissues (Figure 12) and the great water-absorbing capacity of the undenatured muscle fibers, the enzyme dissolved in the rehydrating liquid is quickly brought into contact with the tissue components *(8)*. Blocks of the dehydrated tissues of comparable size to those of raw *semitendinosus* tissue were immersed in distilled water in which the test enzyme was dissolved. This technique has the additional advantage of affording more precise control over the over-all enzyme activity level by varying the concentration of enzyme (expressed in per cent as grams of the enzyme in 100 cc. of rehydrating water) and the rehydrating time.

All samples were histologically processed without trimming or further manipulation of any kind except those powdered with the enzyme, in which case they were first washed in water to remove the adhering enzyme preparation. Standard celloidin embedding procedures were followed. The treated tissues were fixed in 10% neutral formalin, cut 12 microns thick and stained with hematoxylin and Van Gieson's stain, except for studies of elastic tissue in which case sections were stained with hematoxylin, orcein and Orange G. Each sample was represented by a number of slides, each con-

[d] Both prepared by Wilson & Co., Inc.
[e] Adolph's meat tenderizer.
[f] Supplied by Rohm and Haas Co., as noted previously.

taining 3 to 5 sections. For most samples both longitudinal and transverse sections of the material were obtained for microscopical examination and analysis.

All experiments were performed at room temperature; no attempt was made to control the pH at which the reactions were carried out.

The number and type of experiments conducted are as follows:

I. Raw fresh beef *semitendinosus* muscle with Rhozyme P-11 or Adolph's meat tenderizer.
By powder: 5, 15, or 30 minutes for each of the two enzymes;
By 10% solution: 5, 15, 30, or 60 minutes for each of the two enzymes.

II. Frozen-dried *longissimus dorsi* muscle with Rhozyme P-11.
1% for 15 minutes or two hours; 5% for 5 or 60 minutes;
10% for 1, 10, or 30 minutes; controls for dehydrated (frozen-dried) and rehydrated (15 minutes in water) tissues.

III. Frozen-dried beef *longissimus dorsi* muscle with either Rhozyme P-11 or Rhozyme concentrated.
Rhozyme P-11: 1% for 15 minutes; 5% for 5 or 60 minutes; 10% for 10 minutes.
Rhozyme concentrated: 1% for 5 minutes; 3% for 5 minutes; 0.3% for 5 or 15 minutes; 0.1% for 15 minutes; controls for dehydrated (frozen-dried) and rehydrated (15 minutes in water) tissues.

A number of photomicrographs were made in order to illustrate the results.

RESULTS AND DISCUSSION

Structural manifestations of enzymatic action in frozen-dried muscle tissue. Figures 12 and 13 represent, respectively, the characteristic structure of frozen-dried pork *Longissimus dorsi* tissue before and after reconstitution by rehydration [cf. Figures 3F and 3E for beef *(8)*]. Comparison of these structures brings out the effectiveness of an enzyme dissolved in the rehydrating medium. The molecules of the enzyme quickly fill the interfibral spaces and become absorbed into the muscle fibers as the latter swell and recover their original diameter (i.e., prior to freeze-drying). During the course of this progressive process there is ample opportunity also for the enzyme to act directly on the connective tissue cells and fibers occupying these spaces.

The samples treated with 0.1% Rhozyme concentrated for 15 minutes had completely normal structures. Signs of enzymatic action first appeared in pork rehydrated with 1% Rhozyme P-11 for 15 minutes (Figure 3). Figures 2 and 3 show an increase in the diameter of muscle fibers (49.1 μ as against 42.7 μ for the control) and a thinning of the sarcolemma, indicating an initial step in its breakdown. Complete dissolution of the sarcolemma was frequently observed. One such example is illustrated by Figure 1, which shows a portion of a muscle fiber being deprived of the sarcolemma as a direct result of the enzyme action. Similar changes were observed in beef *longissimus dorsi* muscle rehydrated in 0.3% Rhozyme concentrated for 5 or 15 minutes. In both pork or beef tissues, however, there was no indication of any changes in the connective tissues.

The pork sample rehydrated with 1% Rhozyme P-11 for 2 hours revealed a weakened staining capacity of the muscle fiber nuclei; other than that, the tissue appeared normal. The measurement of muscle fiber diameter did not show a significant increase over that of the previous sample (1%, 15 minutes). This seems to indicate that at this concentration, the enzyme activity advanced no further than that already described.

Further action of the enzyme was observed in the pork tissue rehydrated

Description of Figures

All figures were photomicrographs taken on 4 × 5 inch Kodak XX-Panchromatic film from 12μ celloidin sections (transverse section in Figures 7, 8, 20, and 21; longitudinal section in the rest) stained with Orcein and Orange G in Figures 18 and 19, with hematoxylin and Van Gieson's stain in the rest. Frozen-dried pork *longissimus dorsi* in Figures 2, 3, 4, 5, 6, 12, 13, 18, and 19; frozen-dried beef *longissimus dorsi* in Figures 14, 15, 16, and 17, raw fresh beef *semitendinosus* in Figures 1, 7, 8, 9, 11, 20, and 21. Magnifications: Figures 1, 2, 3, 4, 5, 6, 7, 8, 18, and 19 = ×225; Figures 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, and 21 = ×53.

Figure 1. A single peripheral muscle fiber from a sample treated with 10% Adolph's meat tenderizer for 15 minutes. Arrow indicates the boundary between sarcolemma intact (right) and sarcolemma-free (left) portions.

Figure 2. Reconstituted from frozen-dried pork by 30 minutes immersion in dist. water.

Figure 3. Rehydrated in 1% Rhozyme P-11 for 20 minutes indicating early enzyme action.

Figure 4. Rehydrated in 5% Rhozyme P-11 for 5 minutes; note disappearance of nuclei.

Figure 5. Rehydrated in 5% Rhozyme P-11 for 60 minutes showing continued swelling of muscle fibers and merging of bare muscle fibers.

Figure 6. Rehydrated in 10% Rhozyme P-11 for 10 minutes, representing the final stage of enzyme action characterized by disappearance of cross-striations and extensive merging.

Figure 7. Muscle fibers at periphery of a sample treated with 10% Adolph's meat tenderizer for 60 minutes: Note intact nuclei of muscle fibers, but none in the endomysial spaces.

Figure 8. Muscle fibers at periphery of a sample treated with 10% Rhozyme P-11 for 30 minutes; enzyme had disintegrated all nuclei of the muscle fibers, but not in the endomysial spaces.

Figure 9. Treated with Rhozyme P-11 powder for 5 minutes; arrow indicates extent of enzyme penetration.

Figure 10. Treated by immersion in 10% Rhozyme P-11 solution for 30 minutes; peripheral muscle fibers were disintegrated to a state of "mushiness" and complete obliteration of endomysial space.

Figure 11. Treated by immersion in 10% Adolph's meat tenderizer for 30 minutes showing a much lesser extent of enzyme action compared to Figure 9 or 10.

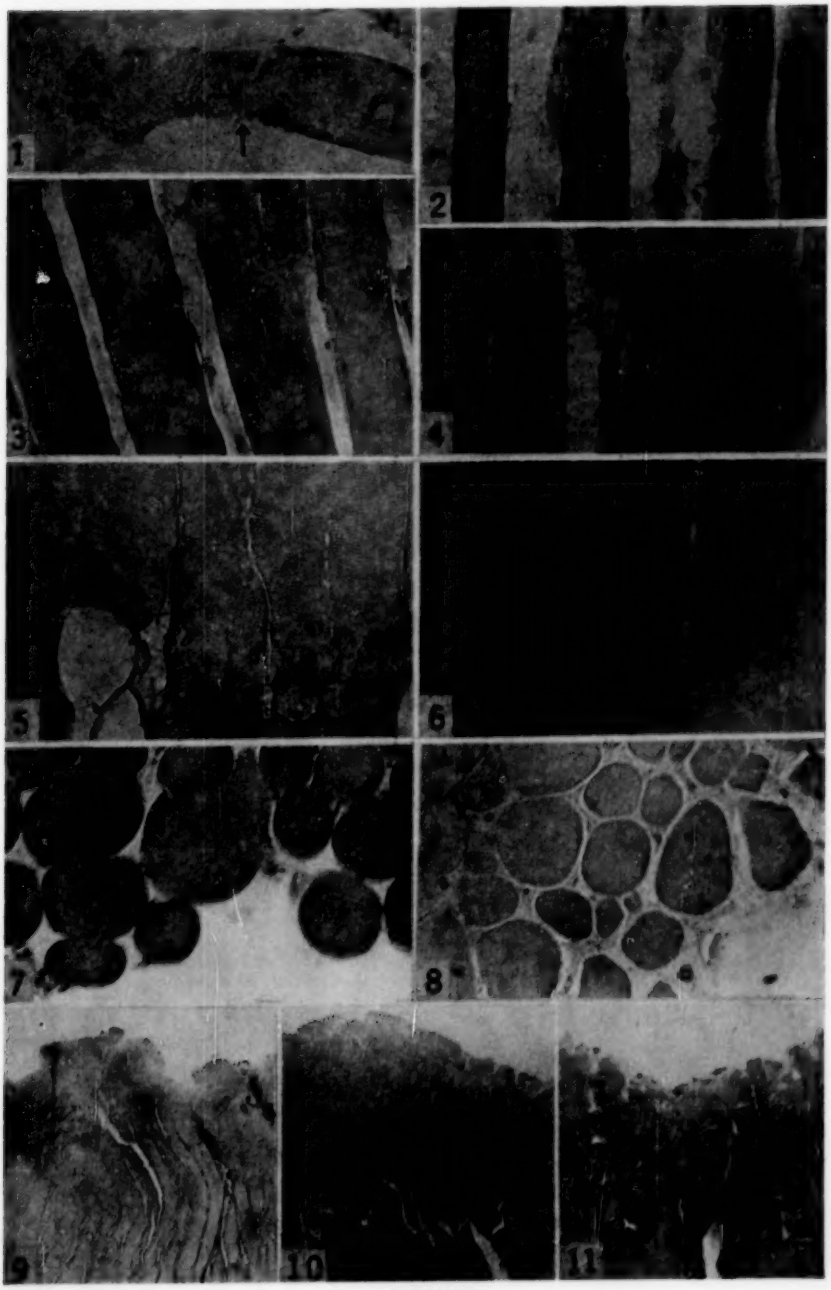

Plate I

Figure 12. Longitudinal section of frozen-dried pork Longissimus dorsi (control).

Figure 13. The above, after rehydration in dist. water for 15 minutes.

Figure 14. Peripheral portion from a sample rehydrated in 3% Rhozyme concentrated for 5 minutes with structures comparable to the stage of enzyme action as shown in Figure 6.

Figure 15. Rehydrated in 1% Rhozyme concentrated for 5 minutes; muscle fibers on left affected to a degree comparable to Figure 4; those on right comparable to Figure 3.

Figure 16. Deeper portion from same sample shown in Figure 14 showing, on right, relatively unrehydrated tissue with distinct nuclei. Note: Figures 15 and 16 illustrate results of occasional incomplete rehydration. In both these samples the center (core) was much less rehydrated than the peripheral tissue. In such cases the core (on right, in both figures) invariably exhibited signs of less enzymatic action, e.g., the presence of distinctly stained nuclei.

Figure 17. Peripheral portion of a sample rehydrated in 10% Rhozyme P-11 for 10 minutes, showing muscle fibers being affected to the same degree as in Figure 6. Note intact collagenous fibers, however.

Figure 18. Rehydrated in water and subsequently stained specifically for connective tissues: Discrete darkly stained elastic fibers against a lighter background of bundles of collagenous fibers.

Figure 19. Rehydrated in 5% Rhozyme P-11 for 60 minutes and similarly stained for connective tissues as above. Note merged enzyme-acted muscle fibers on left; structurally intact collagenous (grayish) and elastic (black) fibers on right.

Figure 20. Treated with Rhozyme P-11 powder for 30 minutes; section parallel to sample's broad surface with muscle fibers oriented perpendicularly to it (in contrast to Figures 9, 10, and 11 from samples with muscle fibers running parallel to their broad surfaces); note equal extent of enzyme penetration to three muscle fiber bundles adjoining a triangular perimysium.

Figure 21. Section from one edge of a sample treated by immersion in 10% Rhozyme P-11 solution for 60 minutes (longest duration used in these experiments) showing one whole muscle fiber bundle being affected by the enzyme. Note intact connective tissue fibers in Figures 11, 20, and 21.

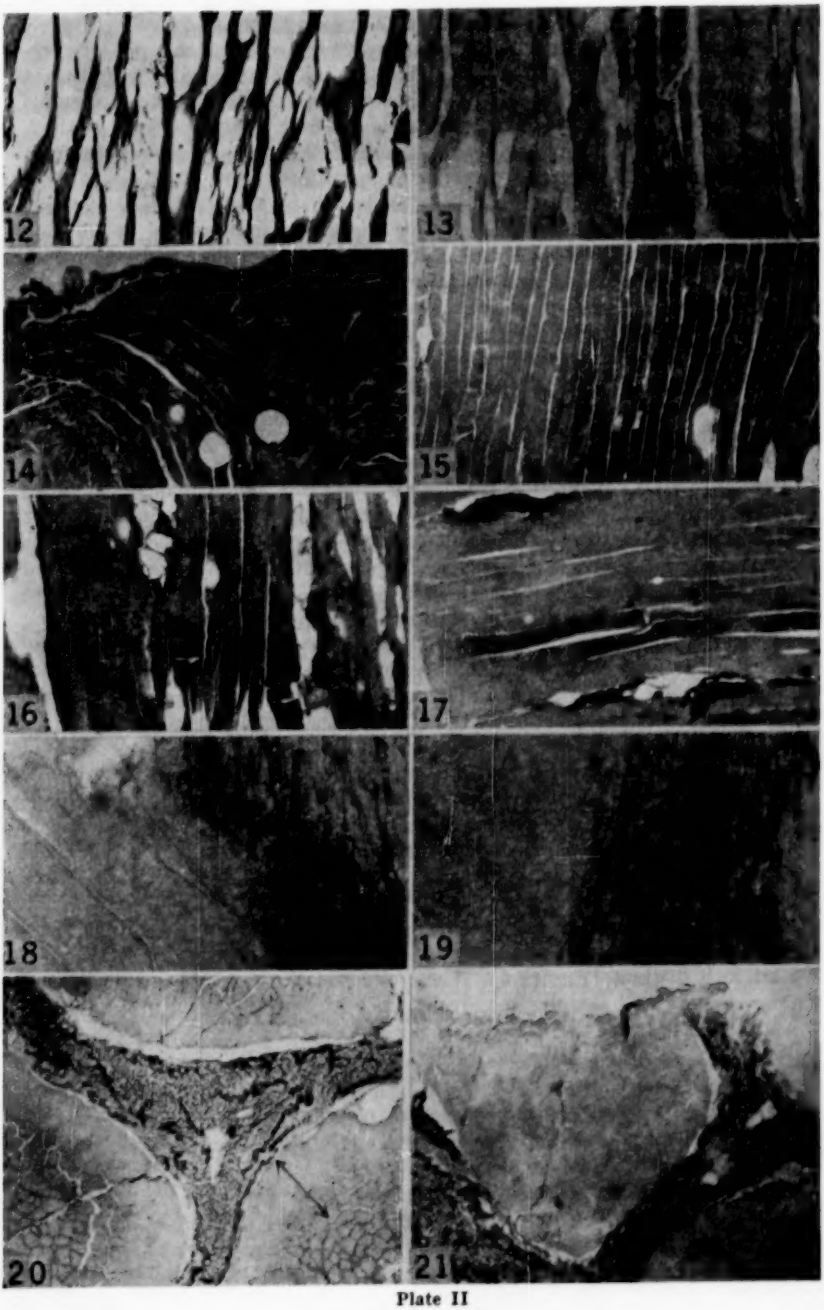

Plate II

with 5% Rhozyme P-11 for 5 minutes (Figure 4) and, to a similar degree, also in beef treated with 1% Rhozyme concentrated for 5 minutes (Figure 15). It was characterized by the disappearance of nuclei and a continued swelling of the muscle fibers, whose diameter averaged 57.7 μ for the sample represented by Figure 4. Again, collagenous fibers were not affected, judging from the way they took the acid fuchsin (red) as well as from their structural integrity.

That the disappearance of nuclei is truly the result of disintegration of their nucleic acid and nucleoprotein content was ascertained by staining the treated tissue with an "over dosage" of hematoxylin (undiluted stock solution for 60 minutes). Even after such prolonged staining, no nuclei of the muscle fibers were evident. In this connection it was interesting to note that Rhozyme has a differential affinity for the nuclei of the muscle cells and those of the cells located in the endomysial and perimysial spaces which consist mostly of fibrocytes, plus certain cells of vascular origin (Figures 4 and 8). Figure 8 was taken from beef *semitendinosus* muscle treated by immersion in 10% Rhozyme P-11 for 30 minutes. The muscle fibers (cross-section) shown therein were in a condition comparable to or slightly further advanced in the degree of enzyme action than those shown in Figure 4. Yet, a few nuclei (dark spots) in the endomysial spaces were clearly stained as contrasted sharply to a complete disappearance of the nuclei from the muscle fibers. The demonstration of such a differential chemical affinity on a structural basis reflects some measure of the degree of sensitivity inherent in the histological technique as a means for studying enzymatic action. The observed differential becomes of even greater significance when one considers that from the standpoint of loci of the two kinds of nuclei, those present in the endomysial spaces have a greater access to the enzyme (Rhozyme) than those within the muscle fibers.

A continued increase in the muscle fiber diameter (59.9 μ) was noted in pork muscle rehydrated in 5% Rhozyme P-11 for 60 minutes (Figure 5). As a result, there was little endomysial space left and the adjacent fibers, now practically bare (due to sarcolemma destruction), thereupon began to merge. Fine collagenous fibrils remained structurally intact and were stained as red as in normal muscle tissue. It was presumed that the sarcolemma had been, by then, completely disintegrated through the continued action of the enzyme. In cross sections, the longitudinal striations of muscle fibers were no longer recognizable although the cross-striations were, for the most part, still distinct (Figure 5). These observations were also made in beef *longissimus dorsi* samples rehydrated in 3% Rhozyme concentrated for 5 minutes (Figures 14 and 16).

The final stage of enzymatic action was found in all samples treated with the 10% Rhozyme P-11, from 5 to 30 minutes. The muscle fibers in these were microscopically extensively merged (Figures 6 and 17) and grossly "mushy." They had lost their individuality, and with this the last vestige and anatomical "land-mark" of muscle fibers, the cross-striations, were gone (Figure 6). The collagenous fibers, on the other hand, persisted as integral structures apparently not affected by the enzyme (Figure 17).

In order to determine whether or not elastic fibers are affected in any

way by the action of Rhozyme, a sample treated with 5% Rhozyme P-11 for 60 minutes and a control (rehydrated in water only) were stained specifically for elastic fibers as outlined earlier. The results of this attempt are shown in Figures 18 and 19. Note that in both photomicrographs the elastic fibers in a perimysium were stained black (with orcein) against a much lighter background of wavy bundles of collagenous fibers (stained with Orange G). The condition portrayed by Figure 19 was typical of samples treated with either Rhozyme or Adolph's meat tenderizer. Therefore, it is quite evident that these enzymes have clear-cut chemical selectivity which is significant. Under the conditions in which our experiments were carried out, they attack muscle fiber protein and nuclei of cells, but not the connective tissue fibers.

Enzymatic action in raw fresh beef semitendinosus tissue with enzymes in solution or spread on. The results from applying the enzymes either in powder form or by immersing the tissue in 10% aqueous solution of the enzymes differed from those described above for frozen-dried samples rehydrated in enzyme solutions in one important respect; namely, penetration of the enzyme into the tissue was limited to the surface of the sample. In all cases studied penetration did not exceed 1 mm. in samples treated for 60 minutes (Figure 21) and was much less in those treated for a shorter time (Figures 9, 10, 11, and 20). Our findings thus confirm an earlier report *(3)*. There is no doubt, therefore, that application of enzymes by either of these two conventional methods for purposes of tenderization of meat is far from adequate, for meat so-treated will have a "mushy" surface but an interior untouched by the enzyme.

The general mode of attack by the papain and fungal enzymes on the raw muscle tissue was very similar, and for both enzymes the same sequence of structural changes as described above generally applied with one exception: the papain preparation while it attacked the nuclei of the cells located in the endomysial spaces, left the nuclei of the muscle fibers intact. Such a characteristic action was clearly illustrated in a sample immersed in 10% of the enzyme for 60 minutes (Figure 7). On the other hand, a sample treated with 10% of Rhozyme P-11 for 30 minutes, as pointed out earlier, exhibited a far greater affinity for the muscle fiber nuclei (Figure 8). This differential specificity of the two enzymes for the two kinds of nuclei can be readily comprehended by examining Figures 7 and 8.

The characteristic appearance of the raw tissue treated by either of the two methods (Figures 9, 10, 11, 20, and 21) convinces one that the action of the enzyme was concentrated on the surface of the sample. This is largely because the muscle fibers, as they absorbed the rehydration liquid, kept on swelling until the endomysial spaces soon became obliterated. As that happened, the enzyme could not penetrate any farther (cf. Figures 9 and 10). This indicates that a longer treatment would only result in overaction (causing mushiness) on the surface of the sample (Figure 10). Note that the extent of enzyme action (arrows) seen in both Figures 9 and 20 was about the same. Figure 20 further illustrates the phenomenon that the enzyme evidently found its way into the tissue through perimysia. The photomicrograph shows a large triangle-shaped perimysium separating three muscle fiber bundles (fasciculi) and only the muscle fibers in contact with

the perimysium were acted on by the enzyme. The affected muscle fibers can be identified by the merging and complete obliteration of the endomysial spaces.

It should be noted that in Figures 11, 20, and 21 some intact collagenous fibers were normally stained, which demonstrates the inactivity of either of the two enzymes on them. The nuclei in the area of enzyme action, on the other hand, were mostly gone.

Further studies are in progress with the use of various levels of enzymes and with correlating measurements of the organoleptic properties of untreated and treated steaks.

SUMMARY

The effect of two proteolytic enzymes of divergent plant origin (a commercial papain preparation and a fungal enzyme preparation) on the structural characteristics of meat was investigated. The enzymes were tested on dehydrated pork and beef *longissimus dorsi* and raw fresh beef *semitendinosus* samples by one of three methods: For the former two muscles, sample blocks were rehydrated in an aqueous solution containing the enzyme; for the raw beef tissue, the enzyme was either spread over the sample or the sample was immersed in a 10% solution of the enzyme. In all experiments the concentration of enzyme and duration of treatment were two variables studied.

It was found that the papain (Adolph's meat tenderizer) and Rhozyme (Rhom and Haas Co.) had very similar effects on the muscle tissue components. Both attack muscle fiber protein, nuclei of muscle fibers and of cells located in the endomysia, but are inactive to collagenous and elastic fibers at room temperatures. The action of Rhozyme on frozen-dried pork and beef *longissimus dorsi* muscles consisted of the following sequence: thinning of sarcolemma, disappearance of nuclei, disintegration of cross-striations in the muscle fibers and merging of bare (sarcolemma-free) fibers to render finally a mushy appearance to the meat. The action is progressive with time, and was satisfactorily studied by rehydrating the tissue in distilled water containing 5% Rhoozyme P-11 for 5 to 60 minutes and comparing the results with similar tissues rehydrated in water only.

The two enzymes when applied to raw fresh beef *semitendinosus* muscle by either powdering or immersion methods proved inadequate and impracticable because of limited penetration of the enzyme into the tissue (less than 1 mm. from the surface in one hour). By these two conventional methods of enzyme application, there were some indications that (a) a greater penetration was obtained in samples with transverse than with longitudinal orientation of muscle fibers with respect to the sample's broad surfaces and (b) slightly greater enzyme penetration was attained in samples treated with solutions than in samples treated by powder application.

The use of structural alterations as criteria for assaying the action of proteolytic enzymes on muscle tissues has been demonstrated. The success of this demonstration depended, to a large degree, on the effectiveness of a technique for rehydrating frozen-dried muscle tissues in an aqueous solution of the enzyme to be tested.

Acknowledgment

The authors wish to express their appreciation and indebtedness to Wilson & Co., Inc. for preparing the dehydrated pork chops and beefsteaks and to Rohm and Haas Co. for providing the Rhozyme preparations used in our experiments.

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THE EFFECT OF WATER IMPURITIES ON THE FLAVOR OF BREWED COFFEE[a,b]

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Coffee is one of the most popular beverages in the United States. The per capita consumption is approximately 13 pounds and the total consumption about two billion, one hundred million pounds. The average coffee drinker consumes three and one-half cups each day or more than 1,250 cups each year.

Coffee has attained its eminent position simply because it tastes good. As a result of much painstaking effort and experience, growers and manufacturers have created the varieties, blends of varieties, roasts and grinds of coffee that yield brews highly acceptable to consumers.

The final step, that of preparing the extract, is a crucial one. Although it is not difficult to make a cup of good coffee, lack of knowledge, indifference, carelessness, and circumstance often act separately or together to prevent the consumer from receiving the full measure of enjoyment that the growers and the manufacturers have struggled so diligently to achieve.

Circumstance, certainly, plays an important role in the success or failure of coffee making. For example, an essential component of coffee is water. Unfortunately, the water we drink and use in the preparation is not simply H_2O, but a complex solution containing many different chemical compounds. The complexity of the solution varies greatly according to the region of origin, the type of storage, the treatment it receives, and many other factors. A vast knowledge concerning types and composition of water supplies throughout the country has become available over the years but relatively little is known about the effect of water impurities on the flavor of beverage coffee.

The object of the present study was to determine what effects impurities normally present in water might induce in brewed coffee.

EXPERIMENTAL

A survey of literature sources and correspondence showed that water supplies are described in terms of some 16 chemical components. These are silica, iron, manganese, calcium, magnesium, sodium, potassium, carbonate, bicarbonate, sulfate, chloride, fluoride, nitrate, dissolved solids, calcium carbonate hardness, and non-carbonate hardness. While this study was in progress an excellent review of the composition and other characteristics of public water supplies became available *(4)*. This publication confirmed our previous choice of components to be studied.

The experimental approach included a determination of taste thresholds for individual components in distilled water according to the method described by Harrison and Elder *(1)* and modified by Lockhart and Stanford *(3)*.

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[a] Presented at the Fifteenth Annual Meeting of the IFT, Columbus, Ohio, June 13, 1955.

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A series of solutions varying in concentration with respect to a single component was prepared. Sodium bicarbonate was used as a source of bicarbonate ions; sodium carbonate, carbonate ions; sodium chloride, chloride ions; potassium chloride, potassium ions; potassium acetate, potassium ions; sodium acetate, sodium ions; magnesium sulfate, magnesium ions; sodium phosphate dodecahydrate, phosphate ions; calcium chloride dihydrate, calcium ions; and ferric sulfate as a source of ferric ions. All chemicals used were of analytical reagent grade. The water was distilled first from a tinned still and then from an all-glass still. All concentrations were prepared in terms of parts per million (p.p.m.) and after preliminary trials were selected so that the odd sample in any test would be identified correctly by more than 33% but less than 100% of the judges.

Each solution and distilled water as a control were presented to members of a taste panel for their differentiation. The experimental design was a randomized triangle test. The solutions, in 25-ml. portions, were served in identical coded beakers. Each set of three consisted either of one sample of distilled water and two of component or two of distilled water and one of component. Combinations were presented randomly and judges were never given any information about the component or the nature of the combination. They were asked only to select the odd sample.

Each taste panel was composed of 18 or more members selected from the departmental staff. Both men and women participated. Some were smokers; others were not. All had previous experience in judging food quality, in threshold testing, and in other flavor studies. Panels were varied in composition.

The tests were conducted in mid-morning and mid-afternoon, at least one hour after meals and in a room designed for work of this kind.

The data obtained from the individual tests are recorded in Table 1. Included in this table are results in terms of percentage correct identifications of the odd sample, and percentage above chance.

As a threshold value became known, a water containing a concentration of the component equal to the threshold value was made up and used for the preparation of coffee beverage. At the same time a control beverage was prepared with distilled water. Triangle difference tests were presented to panels in the manner described above. If the panel could not differentiate the odd sample with statistical significance, the component concentration was increased stepwise until the panel was able to make a significance differentiation. In some cases hedonic scale scores *(5)* were also recorded.

The coffee used was one selected from several as an acceptable brand. It was a Silex-grind type and vacuum-packed in one-pound cans. Silex vacuum coffee makers were employed in the preparation of brews. The brew was prepared generally in 10-cup quantities. The proportion of coffee to water was one standard measure (equivalent to two level tablespoons) per six ounces of cold water. The water was measured into the lower bowl of the maker and heated to boiling. At this point the upper bowl containing the coffee was inserted into the lower. After the water had risen into the upper bowl, the suspension was stirred for 30 seconds and held on the hot plate for a total of 2 minutes. The maker was removed to a cool surface to bring the extract down into the lower bowl as rapidly as possible. This generally required less than one minute.

The coffee was maintained at a temperature of approximately 180°F. (82°C.) until served. It was served in 25-ml. portions. Small amounts of cream and sugar were permitted if the beverage were normally consumed with these optional ingredients. No coffee was held for longer than 30 minutes; fresh beverage was prepared as required.

Data relating to the ability of panels to detect added components in coffee are presented in Table 2.

RESULTS AND DISCUSSION

The data obtained from experiments on threshold testing of ions in water solution were plotted on logarithmic probability paper according to the method of Harrison and Elder *(1)*. A line was drawn visually through the points for each set and the concentration corresponding to a probability of 50% above chance taken as the threshold value. A typical result from the calcium data is shown in Figure 1. A summary of the

TABLE 1

Data for the measurement of taste thresholds of ions in water solution

Ion	Conc. p.p.m.	Separations		Percentages	
		Total	Correct	Correct	Above[1] chance
HCO_3^-	125	20	8	40.0	9.5
	250	20	9	45.0	17.5
	500	20	11	55.0	32.5
	1000	20	15	75.0	62.5
$CO_3^=$	31	20	13	65.0	47.5
	62	20	14	70.0	55.0
	125	20	16	80.0	70.0
	250	20	18	90.0	85.0
Cl^-	75	20	11	55.0	32.5
	125	20	12	60.0	40.0
	250	20	15	75.0	62.5
	500	20	14	70.0	55.0
Ca^{++}	62	20	9	45.0	17.5
	125	20	13	65.0	47.5
	188	20	17	85.0	77.5
	250	20	18	90.0	85.0
Fe^{+++}	1	20	7	35.0	2.5
	2	20	7	35.0	2.5
	4	20	8	40.0	9.5
	8	20	10	50.0	25.0
	12	20	15	75.0	62.5
$PO_4^{\equiv}$	10	20	7	35.0	2.5
	50	20	13	65.0	47.5
	100	20	12	60.0	40.0
	150	20	13	65.0	47.5
	250	20	16	80.0	70.0
Mg^{++}	50	20	8	40.0	9.5
	75	20	10	50.0	25.0
	100	20	15	75.0	62.5
	125	20	14	70.0	55.0
K^+ in KCl	250	20	12	60.0	40.0
	500	20	15	75.0	62.5
	625	20	16	80.0	70.0
	750	20	17	85.0	77.5
K^+ in KAc	340	20	9	45.0	17.5
	400	20	10	50.0	25.0
	500	19	11	57.9	35.0
	600	19	12	63.2	44.7
	750	20	16	80.0	70.0
Na^+	75	20	9	45.0	17.5
	100	20	12	60.0	40.0
	150	20	12	60.0	40.0
	200	20	16	80.0	70.0

[1] Calculated from formula: % above chance is equal to 3/2 (% observed—% expected).

TABLE 2

Data relating to the effect of ions on the flavor of coffee beverage

Ion	Conc. p.p.m.	Separations		Hedonic scores[1]	
		Total	Correct	Control	Test
$CO_3^=$	55	20	5	5.5	6.3
	100	20	8	3.9	6.0
	125	20	16[2]	4.2	5.0
HCO_3^-	1000	20	16[2]	5.3	5.0
Cl^-	225	40	12	4.8	5.6
	250	20	7	3.5	4.3
	300	20	4	5.0	4.0
	400	20	12[2]	4.3	5.0
Mg^{++}	110	20	9	....	....
	200	20	12[2]	....	....
K^+	340	20	9	....	....
	375	20	9	....	....
	410	20	11[2]	....	....
Fe^{+++}	10	18	11[2,3]	....	....
Ca^{++}	125	20	10	....	....
	250	20	8	....	....
	300	20	11[2]	....	....

[1] Calculated from correct separations only.
[2] 11 or more correct separations, significant, $p < 0.05$.
[3] See discussion on effect of iron in coffee.

threshold concentrations and concentrations giving detectable effects in coffee beverage is shown in Table 3. These results have been calculated in terms of both ions contributed by the salt because different thresholds were obtained for several ions due to the nature of the accompanying ions.

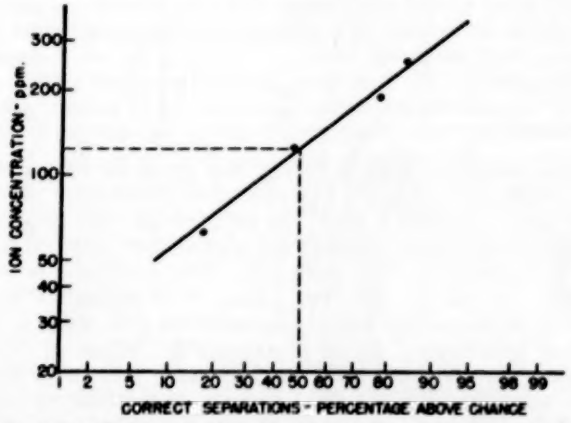

Figure 1. Threshold determination of calcium ion in distilled water. Triangle test. Concentrations of calcium against percentage of correct separations above chance.

TABLE 3

Threshold concentrations of ions in water solution and concentrations in coffee beverage

Ion	Threshold in water p.p.m.	Concentration detectable in coffee beverage p.p.m.
$NaHCO_3$		
Na^+	290	377
HCO_3^-	770	1000
Na_2CO_3		
Na^+	34	96
$CO_3^=$	44	125
Na_3PO_4		
Na^+	75	....
$PO_4^{\equiv}$	105	....
NaAC		
Na^+	140	....
Ac^-	360	....
NaCl		
Na^+	135	258
Cl^-	210	400
KCl		
K^+	340	410
Cl^-	310	450
KAc		
K^+	680	....
Ac^-	1020	....
$CaCl_2$		
Ca^{++}	125	300
Cl^-	222	530
$MgSO_4$		
Mg^{++}	100	200
$SO_4^=$	400	800
$Fe_2(SO_4)_3$		
Fe^{+++}	10	10
$SO_4^=$	25	25

In these cases one must hesitate to say that either of the paired ions is exerting an effect unsupplemented by some effect of the other. For example, five of the salts contained sodium. In combination with bicarbonate the concentration of sodium at threshold was highest, 290 p.p.m. It was lowest, 34 p.p.m. in combination with carbonate. Concentrations in other combination were not higher than 140 p.p.m. It is impossible to say that differentiation of the sodium bicarbonate solutions from water is due either to sodium or bicarbonate. In all probability the action of these ions is supplemental. On the other hand, one can infer that the differentiation of sodium carbonate solutions from water is due almost entirely to the presence of the carbonate ion. In the threshold solutions of carbonate and bicarbonate the ratio of sodium concentrations is approximately 1:9. A

similar analysis might be made of the results that are shown for compounds containing potassium, chloride, acetate, and sulfate ions.

As might have been expected, in those instances in which coffee beverage was prepared with water containing ions at or near their threshold concentrations, no detectable difference in flavor was observed. Increases in concentration ranging from 20% in the case of ions in potassium chloride to 180% in the case of ions in sodium carbonate were required to induce effective changes in the taste of the beverage.

Iron is a special case. Preparation of coffee with iron at 10 p.p.m. yielded a beverage that was differentiated not because of flavor changes but because of changes in color or appearance particularly when cream was added. Although this point has not been investigated, it may be conjectured that the iron combines with polyphenolic components of the extract to produce typical greenish colors. A more detailed qualitative study of this effect indicated that when iron is added to coffee with cream at levels not exceeding 4 p.p.m. the mixtures have a normal appearance if not compared against controls containing no iron. At levels between 4 and 7 p.p.m. there might be some question about acceptability. At levels above 7 p.p.m. a very definite unpleasant greenish cast was readily perceptible. When mixtures containing iron are compared side by side with those containing none, differences are apparent even at an iron concentration of one p.p.m.

Because of limitations associated primarily with panel composition and bias, no attempt was made to determine precisely the relative acceptability, like or dislike, for the members of a pair of coffees when significant differences were obtained. The general observation, however, is that the presence of ions aids in the preparation of a more acceptable brew. Previous evidence *(2)* which still requires confirmation has also indicated that this may be so.

The major objective of this study was to determine whether concentrations of ions at levels normally found in public water supplies have an effect on the flavor of brewed coffee. Table 4 compiled from data recently available *(4)* shows the ion composition of typical water supplies throughout the country. An effort was made to locate areas having water supplies that contained high concentrations of specific ions because no supply contained high concentrations of all ions. Many of the areas have supplies that contain relatively low concentrations of all ions. The bicarbonate appeared to be the ion present in all waters in highest concentrations. A very high value for this ion was 336 p.p.m. This value is well below that found for the threshold (770 p.p.m.) and should not affect the taste of coffee beverage. Iron and carbonate, although having the lowest threshold values, are found in almost negligible concentrations in water supplies. A high concentration of iron was 0.56 p.p.m., found in Sarasota water. It cannot have any effect on the taste of water or coffee and should not produce detectable color changes. Occasionally, as in the case of sulfate, sodium and chloride, there are concentrations high enough to be taste factors. For example, in the Sarasota water the concentration of sulfate is reported as 817 p.p.m. and that for sodium, 530 p.p.m. These values are higher than those for the corresponding thresholds in water and the detectable limit in coffee beverage. In the Galveston water the concentration of chloride is reported as 422 p.p.m. Again this value is higher than threshold for water and the

TABLE 4

Analyses of water supplies used by large cities in the United States

Component	City											
	Boston, Mass.	New York, N. Y.	Chicago, Ill.	Los Angeles, Calif.	San Francisco, Calif.	Indianapolis, Ind.	Cleveland, Ohio	St. Louis, Mo.	Kansas City, Kan.	Galveston, Texas	Sarasota, Fla.	Pittsburgh, Pa.
Iron	0.10[1]	0.03	0.09	0.04	0.02	0.11	0.12	0.01	0.01	0.0	0.56	0.3
Calcium	4.0	13.0	39.0	25.0	1.1	67.0	39.0	23.0	75.0	30.0	14.0	60.0
Magnesium	0.4	4.3	10.0	5.0	1.4	20.0	7.3	9.7	22.0	9.7	0.3	18.0
Sodium	1.8	3.0	3.3	34.0	0.1	6.2	8.7	[33.0	59.0	[351.0	530.0	[49.0
Potassium	0.7	1.4	0.7	4.0	....	1.6	1.3		5.6		16.0	
Carbonate	0.0	0.0	0.0	2.0	0.0	0.0	0.0	13.0	0.0	0.0	0.0	0.0
Bicarbonate	7.0	36.0	132.0	138.0	7.0	206.0	103.0	20.0	237.0	336.0	161.0	17.0
Sulfate	5.6	20.0	23.0	23.0	1.6	67.0	30.0	109.0	172.0	1.0	817.0	248.0
Chloride	3.4	5.8	7.2	17.0	1.0	10.0	20.0	17.0	29.0	422.0	168.0	58.0

[1] All data given in p.p.m.

detectable limit in coffee. However, these are isolated conditions and although important locally, affect only small groups of beverage consumers. Large cities either secure their supplies from sources that are naturally quite pure or finish raw waters by methods that remove or reduce materially concentrations of ions to innocuous levels.

It may be concluded, therefore, that except for local conditions and isolated cases, the inorganic components of water supplies studied to date should not affect the taste of brewed coffee. There remains still the problem of determining by similar techniques the effect of dissolved gases, such as ammonia, sulfides and chlorine, and in addition, organic materials that may form an important odor fraction of the dissolved solids. This work is now in progress.

SUMMARY

Taste thresholds of a number of inorganic ions found in public water supplies have been measured. Coffee prepared with water containing individual ions at threshold levels could not be differentiated from coffee prepared with distilled water. Coffee prepared with water containing higher concentrations of ions could be differentiated. The levels at which differentiation was possible were found.

Except for some minor local supplies, the concentrations of inorganic components studied should not affect the taste of brewed coffee.

Acknowledgment

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THERMAL RESISTANCE DETERMINATIONS IN STEAM USING THERMAL DEATH TIME RETORTS*

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(Manuscript received June 21, 1955)

A method for the determination of the resistance of bacterial spores at temperatures above 212°F. in steam under pressure was first applied by Richardson in 1926, who, according to Townsend, Esty, and Baselt *(14)* developed thermal death time retorts for heating sealed thermal death time tubes. A thermal death time can of small dimensions, giving rapid heating of the contents, was described by Townsend, Esty, and Baselt *(14)*, Braun *et al. (4)*, and Pilcher *(7)*. Although the thermal death time retort system has been used in a number of laboratories over a period of years, the published descriptions of this equipment are somewhat meager and incomplete. It is the purpose of this paper to describe the construction and operation of a thermal death time retort system for use with thermal death time cans together with modification for use with the unsealed cotton plugged tube procedure described by Schmidt *(9)*.

EXPERIMENTAL

Description of equipment. The equipment constructed in this laboratory is similar in some basic essentials to units used in other laboratories. However, it differs in that it was designed and constructed for use either with thermal death time cans or with cotton plugged tubes cooled under pressure. Thermal death time cans are used to determine the resistance of spores in a product based upon product incubation for recovery of survivors. The plugged tube procedure is used to determine resistance in either product or a standard substrate such as phosphate buffer based upon recovery of the survivors in subculture media. The advantages of the plugged tube procedure for the recovery of survivors where subculture is desired have been discussed by Schmidt *(9)*.

To assist in describing this equipment and in illustrating its operation, reference is made to Figures 1, 2, and 3. Figure 1 is a photograph of the six retort unit in our laboratory and serves to give an over-all concept of the unit as related to the operator. Figure 2, for convenience of representation, shows a two retort unit only. The specifications listed in the following description are those for a six retort unit. However, with the exception of some of the manifold pipe sizes and the length of the ballast tank, they would apply to any single or multiple set-up.

As shown in Figure 2 the unit consists basically of a supporting stand (1), the ballast tank (3), the retorts (2), a temperature control system and adequate facilities for supplying dry steam, water, air (controlled) and proper drainage.

The stand is made of 2×2 inch angle iron and supports the ballast tank, the retorts and the manifold piping of the facilities. It also supports a 1×12 inch work shelf *(18)*. The ballast tank is made of 8-inch pipe, 12 feet long, and has a capacity of about 5 cubic feet. It is suggested that this tank be increased to 12 inches in diameter which would provide a 9.5 cubic foot capacity. For less than 6 retorts, this tank could be correspondingly shorter; however, it should contain not less than 3 cubic feet if a single retort is used.

The ballast tank is also equipped with an indicating thermometer (14), a ⅛-inch bleeder cock (16), and a ¾-inch gauge safety valve (17). The vent line (15) for

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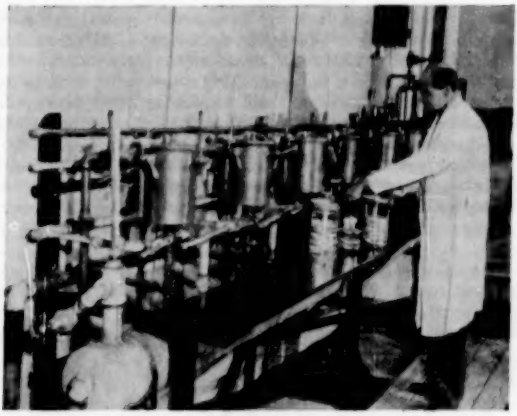
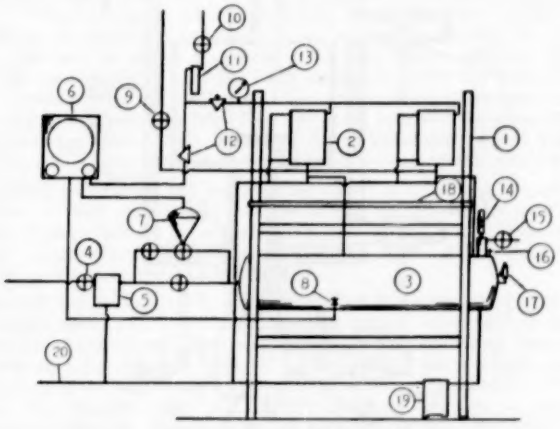


Figure 1. Six unit thermal death time retort system.

purging the tank is a 1¼-inch line with a gate type valve control. The retorts will be described in detail later. The temperature control system is a Minneapolis-Honeywell Electronic controller (range 150 to 300°F.) (6) activated by a thermocouple (8) located in the ballast tank.



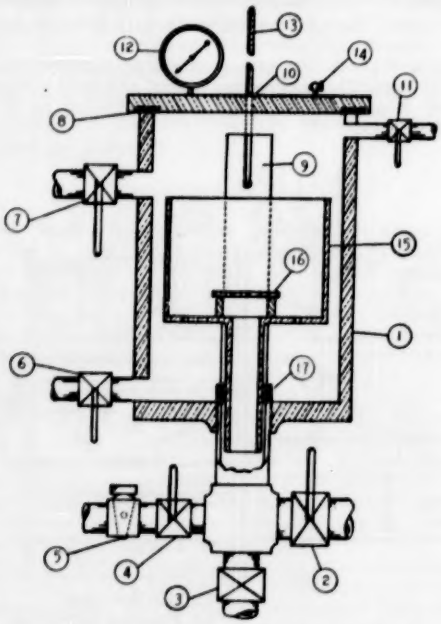
1. Stand
2. Retort
3. Ballast tank
4. Steam line, 1"
5. Steam separator and trap
6. Temperature control, pneumatic
7. Regulating valve, reverse acting (air to open)
8. Thermocouple, control element
9. Water line, 1¼"
10. Air line, 1"
11. Air line filter
12. Pressure regulators
13. Pressure gauge
14. Thermometer
15. Vent line
16. Bleeder cock, ⅛"
17. Pop safety valve
18. Work shelf
19. Trap
20. Drain line, 2"

Figure 2. Thermal death time retort system showing general arrangement of components.

This controller operates a ¾-inch air operated, reverse acting, throttling type valve (7). Steam is supplied by a 1-inch line (4) at 80 to 100 p.s.i. line pressure. In order to insure dry steam a combination steam separator and trap (5) is located in the steam supply line just before the regulating valve. The retorts are supplied by steam from the controlled ballast tank and experience has shown that there is no appreciable difference between the temperature existing in the ballast tank and in the retort proper. Water for cooling is supplied by a 1¼-inch line (9). Air for the control instrument and pressure cooling is supplied by a 1-inch line (10). Air for pressure cooling is regulated by the upper pressure regulator (12) as indicated by gauge (13). The ballast tank is also equipped with a trap (19) for removing condensate which in turn drains into the common drain (20) which serves the retorts and the separator.

To make the unit ready for operation, the controller (6) is set to the desired temperature, the vent line (15) is opened, and steam is admitted. When the temperature indicated by the thermometer (14) in the ballast tank reaches 220°F. and at least 2 minutes have elapsed after steam is turned on, the vent is closed and the unit is ready for use.

Figure 3 shows the thermal death time retort in detail with the modifications



1. Retort body (bronze)
2. Steam inlet from ballast tank, 1"
3. Bottom drain, ¾"
4. Water inlet, 1½"
5. Water flow control valve, ½"
6. Side drains, 1½"
7. Overflow, ¾"
8. Cover gasket, rubber
9. Thermometer well
10. Retort cover (bronze)
11. Air inlet, ¼"
12. Pressure gauge
13. Thermometer
14. Bleeder cock, ⅛"
15. Tube adapter
16. Adapter steam baffle
17. Adapter support

Figure 3. Vertical cross-section of thermal death time cell showing details of construction.

necessary for the plugged tube technique. The retort body (1) is 5½ inches in diameter and 8½ inches deep inside. It is of cast high tensile bronze with walls ⅜-inch thick. The cover (10) is of the same material, with a rubber gasket (8) and is held by 3 cam type fasteners. The thermometer well (9), an integral part of the retort body, is semi-cylindrical and occupies a position at the rear of the retort. The retort is equipped with an indicating thermometer (13) in this well, and also a pressure gauge (12) and a bleeder cock (14) in the cover. All operating valves are lever type, quick opening gate valves located in positions to give convenient and fast operations. Each facility for all retorts leads to its common manifold line. The steam supply, of course, is the ballast tank common to all retorts.

The hook-up for thermal death time can tests consists of a 1-inch steam line (2) coupled closely to the ballast tank, a ¾-inch bottom drain (3), a ½-inch water inlet (4) and a ¾-inch overflow (7) centered approximately two inches below the lip of the retort body. In practice it has been found that a 10-second venting period through the over-flow will purge the retort of air and that the retort come-up-time will be 15 to 20 seconds depending upon the operating temperature being used.

The modifications necessary to permit the use of the plugged tube technique require the addition of a ½-inch side drain (6), a ¼-inch air inlet (11) with a controlled air supply for pressure cooling, a ½-inch petcock valve (5) in the water line for controlling the rate of flow of the cooling water and an adapter (15) for holding a suitable rack for the test tubes. The adapter is a cylindrical vessel for holding the tube rack with a stand pipe which serves both as an inlet for steam and water and as a support. The adapter body is made of 4½-inch brass tubing with a ⅛-inch wall and is 3⅝ inches deep. The flat bottom of the adapter is also ⅛-inch brass stock turned to fit and brazed into the tubing. There are four ¼-inch holes in this bottom for draining purposes. The stand pipe is a ¾-inch brass tube with ⅛-inch wall approximately 5 inches long. This pipe is brazed into the bottom of the adapter. By turning down this supporting tube slightly it can be made to fit into the 1-inch bottom inlet of the retort and by means of a collar (17) fitted with a set screw the height of the adapter can be adjusted so that the lip is about level with the bottom edge of the side drain as shown in Figure 3. A baffle (16) serves as a steam spreader for providing uniform heating throughout the tube load and also prevents the disturbance of the tubes by the entering steam. This baffle is a 2-inch brass disc ⅛-inch thick with six 3/16-inch holes and is supported ½-inch above the bottom of the adapter.

The procedure for venting and bringing the retort to operating temperature is the same with tubes as with cans except that the side drain (6) is also used for venting to insure that all air is purged from the bottom part of the retort. Also between runs the side drain (6) is opened to secure better draining of the retort which is somewhat delayed by the position of the adapter tube.

At the end of the heating cycle, pressure cooling is accomplished quite simply by closing the bleeder cock (14) in the cover, opening the air valve (11) to introduce air at a pressure corresponding to the steam pressure at the operating temperature and introducing cooling water through valve (4). As shown by thermocouple measurements cooling is quite rapid, the temperature within the tube falling from 250-260°F. to 212°F. in 0.2 minute. The petcock valve (5) serves to regulate the flow of water into the adapter so that flooding of the closed retort system does not take place during the cooling time.

Gas closure of thermal death time cans. As an alternative to vacuum closing, the thermal death time cans are closed on a motorized Dixie steamer with undercover gassing, using a magnetized chuck to hold the covers. A modification of a commercial undercover gassing device is used. In use the inoculated filled can is placed on the base plate and the cover on the chuck; with a controlled flow of gas the can is lifted into place and the cover seamed in the usual manner. Tests have been made of the efficiency of headspace gas replacement by closing cans with CO_2 gassing, withdrawing the total gas in the can by vacuum, absorbing the CO_2 and determining the residual volume of air. Using a fill of 15 ml. of water which would leave a headspace volume of 4 ml., 20 determinations of residual air varied between 0.05 and 0.10 ml. with a mean value of 0.06 ml. This air replacement is equivalent to what would be achieved by a 29.5 inch vacuum. Using a fill of 9 grams of corn and 4.5 ml. of brine, 8 determinations of

residual air varied between 0.10 and 0.20 ml. with a mean value of 0.14 ml. This air replacement is equivalent to that produced by a 29.25 inch vacuum.

Determination of correction for lag during come-up and cooling. The importance of adequate correction factors in thermal death time work has been emphasized by Sognefest and Benjamin *(11)*, Reynolds *et al. (8)*, and Anellis, Lubas, and Rayman *(1)*. Correction factors for come-up and cooling in thermal death time cans and plugged tubes are determined in the following manner. In thermal death time cans, heat penetration measurements are made with an Ecklund *(5)* type thermocouple using the size normally used for a 202 diameter can. In order to bring the thermocouple tip to the center of the vertical axis of the can, a threaded adapter is soldered to the bottom of the can at the center of the flat portion, and a hole large enough to accommodate the thermocouple is drilled through the can bottom. The thermocouple is then inserted before filling the can with product. After closing, the can is placed in the retort and connected to a recorder by a lead constructed of 24-gauge copper and constantan wires having the proper plug-in connectors to fit the thermocouple and recorder. The copper and constantan wires were each separately insulated by an asbestos covering and the lead between couple and recorder brought out over the top edge of the retort. With the type of gasket used no significant leakage of steam occurred when the top was locked in place.

To determine the heat penetration in plugged tubes, a thermocouple constructed at the end of a length of 24-gauge duplex vinyl coated copper and constantan wires is used. The thermocouple tip is inserted in the center of the one ml. of product in the tube and the flexible wire brought out over the top edge of the retort. A plug-in connector is used to attach the couple to the recorder.

The heat penetration data on either cans or tubes are obtained using a high speed single point strip-chart recorder, running at one linear inch of chart per minute, with a pen to trace the heating and cooling time. Temperatures are read from the chart at 0.2 minute intervals and the lethality of the product come-up and cooling time determined

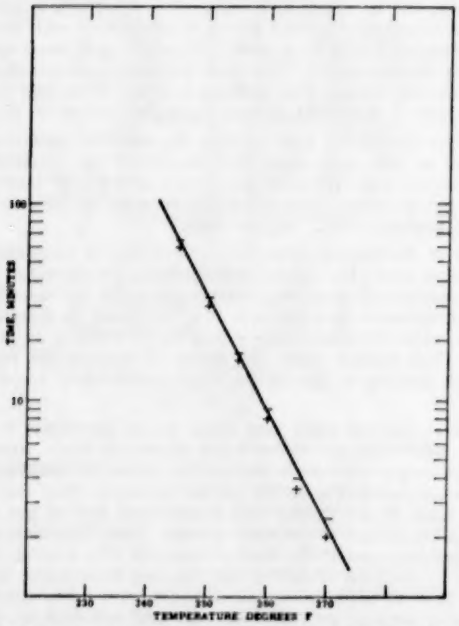


Figure 4. Thermal death time curve for 1,000 spores of N.C.A. 1518 in cream style corn determined in thermal death time cans. ($F = 33$; $z = 17$)

by the graphical method, Bigelow *et al.* *(3)*. The correction is then obtained by subtracting the time representing the lethality of come-up and cooling from the total come-up time. Rather than subtracting the correction from the total exposure time, the correction factor is added to the desired heating times based on instantaneous product come-up and cool. In this way all corrected exposure times are expressed in an even number of minutes or a prescribed fraction thereof. This makes it possible to compare more easily the resistance of suspensions in terms of survival and destruction time in products with different correction factors.

RESULTS

The general procedure of an experiment will be illustrated with the determination of the thermal resistance of a spore suspension of N.C.A. 1518 in cream style corn and in phosphate buffer.

The resistance in cream style corn is determined in thermal death time cans followed by product incubation at 130°F. Thermal death time cans are inoculated with 0.1 ml. of suspension containing the desired number of spores, 1,000 in the case used for illustration. Cream style corn which has been unprocessed in frozen storage is used as substrate. The thawed corn is weighed into the thermal death time cans, using a fill of 13 g., and the cans are then closed with undercover gassing as previously described. After thorough shaking, the cans are exposed in the retort in lots of 10 cans for each time interval at each temperature. The time intervals of exposure are adjusted for the lag correction and the retort operated as previously described. Following processing the cans are incubated for two

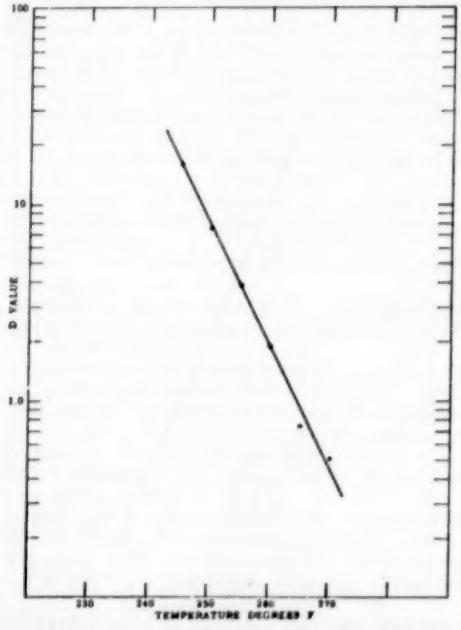


Figure 5. Thermal resistance curve in cream style corn for spores of N.C.A. 1518 from data obtained in thermal death time cans. ($D_{250} = 7.8$; $z = 16.5$)

weeks at 130°F., then opened and examined for spoilage. Typical results are illustrated by the thermal death time curve in Figure 4, showing the survival and destruction points for this suspension at temperatures between 245°F. and 270°F. From the spoilage data at each temperature, the corresponding D values may be calculated by one of several methods suggested: Stumbo *(12)*, Stumbo, Murphy, and Cochrane *(13)*, Schmidt *(10)*. Figure 5 shows the plot of the thermal resistance curve, that is, the plot of the logarithm of D versus temperature.

The resistance in phosphate buffer is determined by introducing 0.1 ml. of suspension into cotton plugged 13 × 100 mm. tubes, followed by 1 ml. of M/15 phosphate buffer. The tubes (10 for each time interval at each temperature) are heated and cooled as previously described. Following cooling the tubes are subcultured by the addition of a medium composed of 1% Tryptone, 0.5% dextrose and 0.5% soluble starch and incubated at 130°F. for six days. Surviving spores of the thermophiles usually germinate within 24-48 hours and additional tubes showing survivors seldom appear after 3 days incubation.

The results of these tests may be plotted either as survival and destruction times to give a thermal death time curve, or D values may be calculated and plotted to give the thermal resistance curve.

Figure 6 shows the thermal resistance curve for this suspension of NCA 1518 in phosphate buffer for temperatures between 245°F. and 260°F.

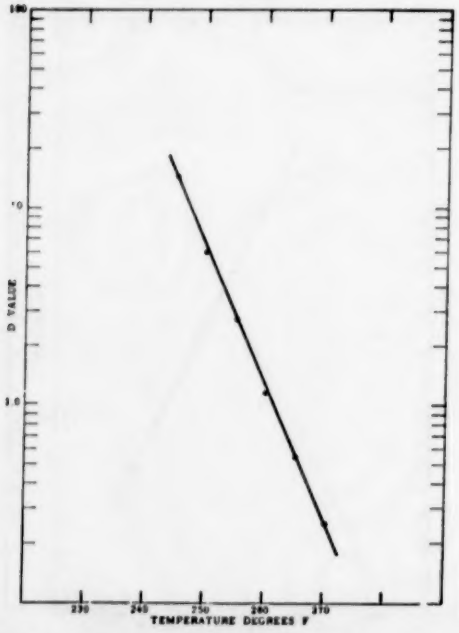


Figure 6. Thermal resistance curve for spores of N.C.A. 1518 in phosphate buffer from data obtained by the plugged tube procedure. ($D_{250} = 6.2$; $z = 14.5$)

The essential linearity of the thermal death time curve and thermal resistance curve is of course merely confirmation of the facts as found by many workers: Bigelow *(2)*; Townsend, Esty, and Baselt *(14)*; Stumbo, Murphy, and Cochrane *(13)*; Pflug and Esselen *(6)*. However, the close fit of the data in the experiments illustrated serves to justify the adequacy of the technique described and its satisfactory operation to temperatures as high as 270°F. Of course, the resistance of the inoculum determines to some degree the upper limits of temperature at which satisfactory results may be obtained. In general the minimum total corrected exposure time giving some survivors must be longer than the come-up and cooling time of the sample either in the can or the tube.

SUMMARY

The design and construction of a thermal death time retort system for use with either thermal death time cans or cotton plugged tubes has been described. The satisfactory operation of this equipment with either cans or tubes to temperatures as high as 270°F. has been demonstrated by the presentation of typical data.

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GROWTH AND PECTOLYTIC ACTIVITY OF SOME TOMATO MOLDS AT DIFFERENT PH LEVELS[a]

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Some molds destroy plant material, especially fruits and vegetables, by the secretion of pectolytic enzymes. Most previous investigations have dealt with commercial preparations of fungal pectic enzymes obtained from mycelial mats and/or cultural fluids. A short review of the available literature on exocellular pectolytic activity of fungi follows.

In 1921, Harter and Weimer *(7)* studied 11 species of the genus *Rhizopus*. All species produced pectinase when grown in a vegetable decoction medium. The amount of pectinase produced varied with the species, even though they were grown under identical conditions. *Rhizopus nigricans* was found to secrete small amounts of pectic enzymes.

In 1939, Proskuriakov and Ossipov *(13)*, used *Aspergillus niger, Aspergillus sp., Mucor sp., Botrytis cinerea* and *Rhizopus nigricans* as the source of pectinase. They indicated that it is the strain rather than the species which is important in selecting an organism for the production of pectolytic enzymes.

Several other investigators *(2, 12)* reported pectolytic activity by the molds *Aspergillus sp., Penicillium sp., Oospora sp., Fusarium oxysporum, Botrytis cinerea, Mucor sp.,* and *Rhizopus nigricans* grown on various plant materials.

It is also well established that the hydrogen ion concentration of a medium exerts a decided effect upon the rate and amount of growth as well as upon many other life processes of the fungi. In 1923, Harter and Weimer *(8)* studied *Aspergillus niger, Penicillium sp., Botrytis cinerea,* and *Fusarium oxysporum* as to growth in vegetable decoction media at different pH levels. They concluded that no growth occurred at pH 2.0 but that at all other hydrogen ion concentrations the same amount of mycelia was produced as long as the medium was not alkaline enough to inhibit growth.

In 1946, Phaff *(13)* examined a strain of *Penicillium chrysogenum* in a synthetic medium. Pectolytic enzymes were produced as soon as the surface of the mat began to form. The secretion continued even after autolysis of the mycelium. This was evidenced when the mycelial weight showed a drop after reaching a maximum value; the pectolytic activity did not decrease.

Many workers *(5, 14)* have reported that the following molds attack tomatoes and tomato products: *Penicillium, Alternaria, Colletotrichum, Fusarium, Mucor, Rhizopus, Aspergillus, Oospora, and Botrytis.*

The present study was undertaken to determine the growth and pectolytic activity of some tomato molds at different pH levels.

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[b] Graduate Fellowship Student.
[c] Professor of Microbiology and Public Health.

EXPERIMENTAL

Twenty-five molds, isolated from tomatoes,[4] were tested for pectolytic activity and amount of growth in canned tomato juice at pH levels of 2, 3, 4.5, 6.0, 6.7, 7.0, and 10.8.

The tomato juice was filtered through double layers of cheese cloth. Different batches were first adjusted to the pH levels of 2.0, 3.0, 4.5, 6.0, 9.0, 11, and 12.0 by addition of 0.1N NCl or 0.1N NaOH. Fifteen ml. portions were then placed in 50-ml. Erlenmeyer flasks and sterilized at 121°C. for 15 minutes. After sterilization, the initial pH levels of 9.0, 11.0, and 12.0 changed to 6.7, 7.0, and 10.8, respectively. The rest of the pH levels, i.e., 2.0, 3.0, 4.5, and 6.0, remained the same.

The molds were grown at room temperature for a period of a week on slants of the sporulation medium formulated by Gailey *et al. (6)*. Two and one-half milliliters of sterile distilled water was added to each slant and the growth loosened gently with an inoculating needle. Five hundredths of a milliliter of the suspension was used to inoculate the tomato juice at the specific pH level. The flasks were then incubated at room temperature for 4 days and the following tests carried out:

(a) One ml. of tomato juice was added to 15.0 ml. of 1.5% pectin N.F.[5] solution and incubated for 24 hours at 30°C. Pectolytic activity by viscosity measurement was determined by the method of Bell, Etchells, and Jones *(1)*.

(b) The amount of mold growth was determined by weighing the mold mats after washing them with distilled water and drying at 60°C. for 10 hours.

(c) The final pH of the tomato juice was determined.

Measurement of viscosity was made at three different times. The weight of mold mats and pH were determined twice.

RESULTS AND DISCUSSION

The pectic enzymes known today are pectin-methyl esterase, pectin-polygalacturonase and the so-called depolymerase. Pectinase is now considered to be an enzyme complex that contains polygalacturonase *(13)*. It is also established that the enzyme polygalacturonase (PG) hydrolizes the 1,4 glycosidic linkages in the polygalacturonic acid chains of pectin and pectinic acids. The enzyme pectin-methyl esterase (PE) catalyzes the hydrolysis of the methyl ester groups in pectinic acids and pectin.

It is well known that the decomposition of polygalacturonic acid chains in the pectin molecule results in loss of viscosity of pectin solutions. These viscosity changes might be caused by the combined action of pectin-methyl esterase and pectin-polygalacturonase. However, according to Kertesz *(9)*, the changes caused by pectin-methyl esterase are very small and can be considered negligible compared with those caused by polygalacturonase. Consequently, it might be logical to consider that the results obtained in this study mostly represent the activity of polygalacturonase rather than the action of pectin-methyl esterase. Yet, as no chemical determination of the enzymes has been carried out, the term "pectinase," designating the action of the complex polygalacturonase-methyl esterase, seems more appropriate to describe the pectolytic activity noted in these experiments. It should be mentioned that the Cup Assay Method described by Dingle *et al. (4)*, was used to determine polygalacturonase activity at the various pH levels selected. The method was not sensitive enough in many instances, however, to detect quantitatively the enzyme activity present.

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[4] Molds isolated from Indiana and Ohio tomatoes in summer of 1953. This is part of a project financed by The Tomato Research Foundation, Elwood, Indiana, at Michigan State College.

[5] Pectin N.F., No. 444D-3 with 9.9% methoxyl content from Sunkist Growers, Ontario, California.

It is recognized that there are limitations to the viscosity method of measuring pectinase activity. Deuel and Weber *(3)* decided that the method is not suited to kinetic investigations. In addition, it does not measure the amount of pectic enzymes present. The application of this method does

TABLE 1

Pectolytic activity (as evidenced by percentage loss of viscosity) of different molds grown in tomato juice at various pH levels for four days.

Name of mold	Percentage loss of viscosity (aver.) pH					
	2.0	3.0	4.5	6.0	6.7	7.0
Aspergillus clavatus	0	86	87	83	83	79
Aspergillus niger	12	91	91	88	88	90
Aspergillus terreus	13	90	90	88	89	90
Botrytis cinerea	19	90	90	88	90	90
Colletotrichum phomoides	11	90	90	88	90	90
Fusarium oxysporum	0	90	90	89	89	83
Fusarium cephalosporium	0	90	90	82	65	53
Mucor globosus						
No. 77	0	90	89	85	87	86
No. 83	0	86	89	88	87	53
Mucor hiemalis						
No. 73	0	89	86	88	73	47
No. 74	0	87	86	89	81	22
Oospora						
No. 94	0	88	90	83	84	60
No. 98	0	89	90	85	85	46
Penicillium cyclopium						
No. 110	0	78	90	90	90	90
No. 121	0	57	89	88	90	90
No. 122	0	82	89	89	90	87
Penicillium oxalicum						
No. 111	0	76	90	90	88	89
No. 116	0	65	89	88	86	85
Penicillium purpurogenum						
No. 113	0	70	87	88	88	87
No. 119	0	87	86	86	83	52
Rhizoctonia solani						
No. 128	18	89	90	91	91	90
No. 140	0	88	89	91	89	90
Rhizopus nigricans						
No. 154	0	88	89	89	90	89
No. 160	0	89	89	89	88	89
Trichoderma lignorum	0	88	89	91	89	89
Uninoculated control	2	3	2	2	2	2

No pectolytic activity was observed at pH 10.8.

point out, however, the comparative pectolytic changes at the different pH levels used.

It may be observed from Table 1 that of all 25 species of molds examined none showed any pectolytic activity at pH 10.8; only 5 species (*Aspergillus niger, Aspergillus terreus, Botrytis cinerea, Colletotrichum phomoides,* and *Rhizoctonia solani* No. 128) showed any activity at pH 2.0. In Table 2,

TABLE 2

Average weight of mats of different molds grown in tomato juice for four days at various pH levels.

Name of mold	Av. weight of mat (mgm.) pH					
	2.0	3.0	4.5	6.0	6.7	7.0
Aspergillus clavatus	....	65	356	340	250	90
Aspergillus niger	....	296	311	313	180	94
Aspergillus terreus	....	293	340	318	223	118
Botrytis cinerea	....	298	340	308	203	84
Colletotrichum phomoides	....	318	312	330	218	121
Fusarium oxysporum	....	135	237	197	160	97
Fusarium cephalosporium	....	160	165	221	95	54
Mucor globosus						
No. 77	....	161	105	98	53	13
No. 83	....	121	110	101	20	13
Mucor hiemalis						
No. 73	....	71	251	262	237	153
No. 74	....	14	178	82	55	15
Oospora No. 94	....	189	272	275	172	114
Oospora No. 98	....	113	228	224	194	113
Penicillium cyclopium						
No. 110	....	204	225	183	151	101
No. 121	....	209	215	202	159	121
No. 122	....	205	232	242	166	109
Penicillium oxalicum						
No. 111	....	165	270	267	232	111
No. 116	....	82	303	301	196	98
Penicillium purpurogenum						
No. 113	....	134	230	265	170	66
No. 119	....	246	298	284	266	111
Rhizoctonia solani						
No. 128	....	285	314	337	278	210
No. 140	....	299	292	319	298	187
Rhizopus nigricans						
No. 154	....	91	200	189	162	108
No. 160	....	160	266	234	146	125
Trichoderma lignorum	....	234	150	139	100	108

No mat observed at pH 10.8.

it can be seen that there is no mat formation at extreme pH levels, i.e., pH 2.0 and 10.8. Therefore, the changes in pectolytic activity as evidenced by the loss of viscosity of the pectin solutions, as well as the weight of mold mats, will be compared throughout the discussion in the range of pH 3.0-7.0.

Effect of pH on percentage loss of viscosity. For the sake of convenience the molds examined were classified in three groups according to differences in per cent loss of viscosity at different pH levels.

Group A. In this group were placed the molds which caused loss of viscosity that did not vary more than 7% throughout the range of pH levels examined, i.e., pH 3.0-7.0. The group included *Aspergillus niger, Aspergillus terreus, Aspergillus clavatus, Botrytis cinerea, Colletotrichum phomoides, Fusarium oxysporum, Mucor globosus* (No. 77), *Penicillium cyclopium* (No. 122), *Rhizoctonia solani* (No. 128 and No. 140), *Rhizopus nigricans* (No. 154 and 160), and *Trichoderma lignorum.*

Group B. In Group B were placed the molds which showed loss of viscosity that was at least 24% lower at pH 7.0 than at pH 6.0. There was slight variation in percentage loss of viscosity from pH 3.0-6.0. The group included *Fusarium cephalosporium, Mucor globosus* (No. 83), *Mucor hiemalis* (strains No. 73 and 74), *Oospora* (strains No. 94 and 98), and *Penicillium purpurogenum* (No. 119).

Group C. In this group were the molds that caused loss of viscosity which was at least 12.0% lower at pH 3.0 as compared with the results obtained at the other pH levels examined. *Penicillium cyclopium* (strains No. 110 and 121), *Penicillium oxalicum* (strains No. 111 and 116), and *Penicillium purpurogenum* (No. 113) fall into this group.

It is emphasized that the foregoing classification of the molds into three groups was purely arbitrary.

Not every species of a genus behaved in the same manner. For example, the mold *Penicillium cyclopium* (No. 110) was classified as belonging to Group C, whereas *Penicillium purpurogenum* (No. 119) was placed in Group B. Even two strains that belong to the same species when grown under identical conditions showed variation, e.g., *Penicillium purpurogenum* (No. 113) and *Penicillium purpurogenum* (No. 119).

When comparing the percentage loss of viscosity at the various pH levels, an arbitrary value of 5% was considered to be insignificant. This was due to the fact that a slight variation, which did not exceed 3.0%, occurred between triplicate determinations. An uninoculated control was used at each pH level to indicate the changes in viscosity in the pectin solution that were not the result of pectolytic activity. The results (Table 1) demonstrate that under no circumstances did the controls show more than 3.0% loss of viscosity.

Effect of pH on weight of mold mats. No mat formation was observed at pH 2.0 and 10.8 in any of the 25 molds examined. Therefore, under the conditions of the experiment these pH levels were demonstrated to be unsuitable for growth.

In the molds *Aspergillus clavatus, Aspergillus terreus, Botrytis cinerea, Penicillium oxalicum* (No. 111), *Penicillium purpurogenum* (No. 113 and 119), and *Rhizoctonia solani* (No. 140) there was a greater decrease in weights of mats at pH 7.0 than at pH 6.7 (i.e., more than 100 mg. differ-

ence). Consequently, pH 7.0 may be considered far from optimum for the growth of these molds.

The pH level at which each mold grew best varied with the individual mold. Some molds grew well over a wide pH level, e.g., *Aspergillus clavatus* and *Colletotrichum phomoides*. Others grew best at pH 6.0, e.g., *Fusarium cephalosporium*, *Penicillium purpurogenum* (No. 113). *Aspergillus terreus* and *Trichoderma lignorum* showed their best growth at pH 4.5 and 3.0, respectively. These pH levels roughly define the optimum pH level for the growth of the molds tested.

Relationship of pH, growth, and pectolytic activity. As to the relation between growth and pectolytic activity as determined by loss of viscosity, the following observations may be made:

(a) When no mat was formed, no pectolytic activity was observed at pH 2.0 and 10.8 except for 5 molds which showed little pectolytic activity at pH 2.0. This was probably due to a small amount of growth of the mold without mat formation.

(b) The amount of growth did not affect the pectolytic activity in the case of the molds that were classified as Group A. The pectolytic activity remained constant throughout the range of pH 3.0-7.0, whereas the weight of mold mats changed (Figure 1).

(c) Molds classified as Group B (i.e., had less pectolytic activity at pH 7.0) showed variable relationships between loss of viscosity and growth of mold. For example, in the case of the mold *Penicillium purpurogenum*

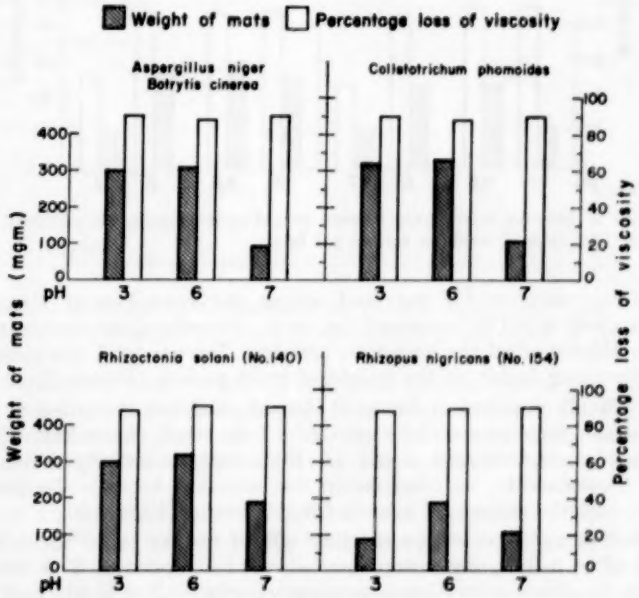

Figure 1. Showing relationship between weight of mold mats and pectolytic activity of group "A" type of molds at various pH levels.

(No. 119), loss of viscosity decreased from 83 to 52% when the pH increased from 6.7 to 7.0. At the same time, the weight of mold mat decreased from 266 mg. at pH 6.7 to 111 mg. at pH 7.0. This might indicate a decrease in pectolytic activity as a result of less growth when the pH changed (Figure 2).

Mucor hiemalis (No. 74) showed 87% loss in viscosity at pH 3.0 whereas the weight of the mat was 14 mg. When the pH increased to 4.5, the weight

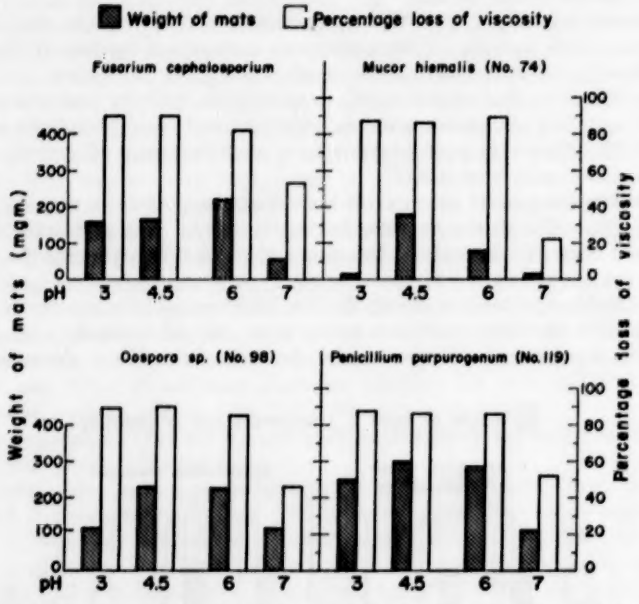

Figure 2. Showing relationship between weight of mold mats and pectolytic activity of group "B" type of molds at various pH levels.

of mat increased to 178 mg. and almost the same loss of viscosity as that obtained at pH 3.0 occurred, i.e., 86%. Therefore, the amount of mold growth did not affect the pectolytic activity. The pH level was most likely the influencing factor on the weight of mold growth (Figure 2).

(d) Molds classified as Group C showed good mat formation at pH 3.0 whereas the pectolytic activity was relatively small. Moreover, when the weights of mats decreased at pH 7.0, the pectolytic activity remained the same. Consequently, no relationship was observed between the pectolytic activity and the amount of growth for this group (Figure 3).

Effect of mold growth on the final pH of tomato juice. Measurements of pH after mold growth were carried out in duplicate. The differences between duplicate determinations never exceeded 0.2 of a pH unit. Thus, when comparing the results in Table 4, an arbitrary value of 0.4 or more of a pH unit was considered to be a significant difference.

TABLE 3

Showing pH at which the various molds grew best in canned tomato juice

Name of mold	pH level at which best growth occurred
Aspergillus clavatus	4.5-6.0
Aspergillus niger	3.0-6.0
Aspergillus terreus	4.5
Botrytis cinerea	4.5
Colletotrichum phomoides	3.0-6.0
Fusarium oxysporum	4.5
Fusarium cephalosporium	6.0
Mucor globosus No. 77 & No. 83	3.0
Mucor hiemalis	
No. 73	4.5-6.0
No. 74	4.5
Oospora No. 94 and 98	4.5-6.0
Penicillium cyclopium	
No. 110	4.5
No. 121	3.0-6.0
No. 122	4.5-6.0
Penicillium oxalicum No.111 and 116	4.5-6.0
Penicillium purpurogenum	
No. 113	6.0
No. 119	4.5-6.7
Rhizoctonia solani No. 128 and 140	6.0
Rhizopus nigricans	
No. 154	4.5-6.0
No. 160	4.5
Trichoderma lignorum	3.0

In all 25 mold cultures examined at pH 2.0 and 10.8, there were no significant changes in the pH level of the tomato juice, after growth of the different molds.

Throughout the range of pH 3.0 to 7.0, the different molds gave variable results. Some molds changed the pH of the tomato juice to the acid side, e.g., *Aspergillus niger,* whereas others caused an increase in the initial pH level, e.g., *Mucor* strains. *Fusarium oxysporum* did not cause any significant changes.

It was observed that at pH 7.0 all the molds classified as belonging to *Group B* increased the final pH level to at least 0.7 of a pH unit. However, not all molds that caused an increase of pH, when the initial pH was 7.0, belong to *Group B,* e.g., *Trichoderma lignorum.*

Some observations of interest. In determining the percentage loss of viscosity, the usual procedure was to add 1.0 ml. of the tomato juice in

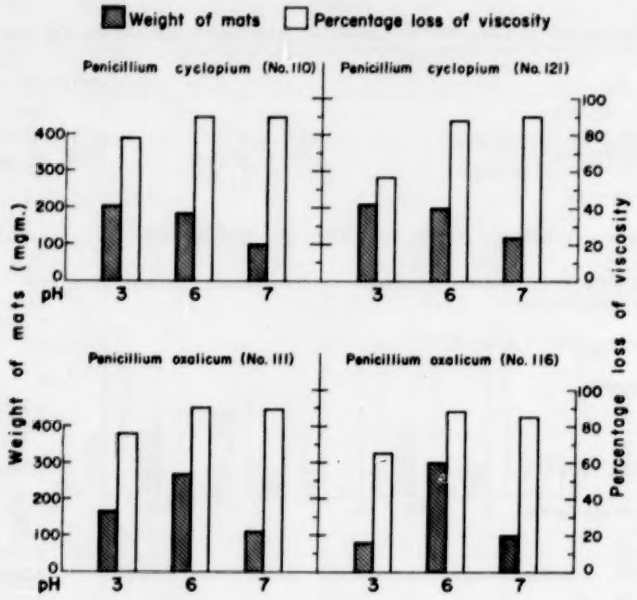

Figure 3. Showing relationship between weight of mold mats and pectolytic activity of group "C" type of molds at various pH levels.

which the specific mold had grown to the pectin solution. Heated inoculated controls were set up by adding 1.0 ml. of heated tomato juice to the pectin solution. Heating the tomato juice was carried out at 80°C. for 30 minutes to inactivate the pectolytic enzymes. After 24 hours' incubation period, the flow time of each mixture was determined and the per cent loss of viscosity was calculated.

With regard to the genus *Mucor* (4 strains examined), loss of viscosity was noticed in the heated inoculated controls. This may have been due to a change in pH of the pectin solution after the addition of the tomato juice in which the *Mucor* molds were grown. There was no difference in the pH of this mixture, however, when compared with heated uninoculated controls. It should be mentioned that the pectin solutions used were buffered at pH 4.5.

It was suggested that the suspended particles in the tomato juice might have had a protecting effect. Consequently, the tomato juice samples were first centrifuged and then heated at 80°C. for 30 minutes. When this was done, activity was observed in the heated inoculated controls in only two of the four strains of *Mucor* examined.

It was considered that the enzyme produced by *Mucor* might be inactivated by increasing the temperature. Therefore, inoculated controls were heated at 90°C. for 30 minutes. Only one strain, *Mucor globosus* No. 83, showed activity in the inoculated heated control. When inactivation was carried out at 100°C. for 30 minutes, activity still occurred.

TABLE 4

Showing the final pH of tomato juice after growth of different molds for four days

Name of mold	Final pH						
	2.0	3.0	4.5	6.0	6.7	7.0	10.8
Aspergillus clavatus	2.0	2.7	4.6	5.1	5.1	6.4	10.6
Aspergillus niger	2.0	2.3	2.7	3.7	5.2	6.1	10.8
Aspergillus terreus	2.0	2.4	2.6	3.4	4.9	6.2	10.7
Botrytis cinerea	2.1	2.4	3.2	3.8	4.9	6.2	10.7
Colletotrichum phomoides	2.1	2.4	2.8	3.5	4.8	6.2	10.7
Fusarium oxysporum	2.1	3.0	4.4	5.7	6.4	6.8	10.6
Fusarium cephalosporium	2.0	3.1	4.5	6.0	6.6	7.7	10.8
Mucor globosus							
No. 77	2.0	3.0	5.5	6.6	7.6	8.1	10.9
No. 83	2.0	2.9	5.4	5.8	7.4	8.1	10.9
Mucor hiemalis							
No. 73	2.0	2.8	6.1	8.0	8.3	8.6	10.8
No. 74	2.0	3.0	5.3	6.2	7.6	8.1	10.9
Oospora No. 94	2.0	3.0	6.4	7.2	8.3	8.5	10.9
Oospora No. 98	2.0	3.1	7.3	8.0	8.3	8.6	10.9
Penicillium cyclopium							
No. 110	2.0	2.5	3.3	3.3	3.3	3.9	10.6
No. 121	2.0	2.8	3.7	3.6	3.8	4.3	10.7
No. 122	2.0	2.7	3.9	3.8	3.9	4.3	10.7
Penicillium ozalicum							
No. 111	2.0	2.6	3.7	3.8	3.8	4.5	10.8
No. 116	2.0	2.9	4.7	4.8	5.0	5.5	10.7
Penicillium purpurogenum							
No. 113	2.0	2.8	4.1	4.7	4.9	5.3	10.9
No. 119	2.0	2.9	6.7	7.3	8.0	8.3	10.7
Rhizoctonia solani							
No. 128	2.0	2.6	4.1	3.8	3.9	4.6	10.7
No. 140	2.0	2.6	4.8	4.0	4.5	5.0	10.9
Rhizopus nigricans							
No. 154	2.0	2.8	5.0	5.3	5.8	7.2	10.6
No. 160	2.0	2.7	4.6	5.2	4.2	4.5	10.9
Trichoderma lignorum	2.0	2.7	5.0	7.0	7.2	8.1	10.7
Uninoculated control	2.0	3.0	4.6	6.1	6.7	8.0	10.7

At all times care was taken to make sure that pectolytic activity was not the result of enzyme production by living molds. This was achieved by streaking an inoculum from the heated controls on the sporulation medium.

It seemed likely that a partially heat resistant factor was excreted by *Mucor globosus* No. 83, which changed the viscosity of the heated controls. A heat stable pectolytic enzyme designated as depolymerase has been

reported by Kertesz *(11)* as occurring in tomato juice. The factor found in this study might therefore be an enzyme similar to depolymerase, or a metabolite excreted by the mold.

On the other hand, when the tomato juice was dialyzed through a semi-permeable membrane, inactivation of the heated controls at 80°C. for 30 minutes was effective. This might indicate that the partially heat stable factor was a metabolite excreted by the mold but dialyzed out. Another possibility might be the presence of certain ions which cause activation of a certain pectolytic factor but lose their influence when dialyzed out. At the present time work is in progress to obtain more information on this factor.

It is of great importance to observe and compare the flow time of heated inoculated controls with the flow time of the uninoculated controls. This procedure is a safeguard against misleading results.

SUMMARY AND CONCLUSIONS

Twenty-five molds were examined for growth and pectolytic activity at pH 2.0, 3.0, 4.5, 6.0, 6.7, 7.0, and 10.8. Pectolytic activity was determined by measuring the percentage loss in viscosity; the amount of growth was measured by weighing the mold mats. From the results obtained the following conclusions may be made:

(a) At pH 2.0 and 10.8, no mat formation occurred and no pectolytic activity was observed except for five molds which showed little pectolytic activity at pH 2.0. This was probably a result of a small amount of mold growth without formation of visible mat.

(b) The pH at which the best amount of growth occurred varied with the species or even the strain of mold.

(c) The amount of growth did not affect the pectolytic activity of the following molds: *Aspergillus niger, Aspergillus terreus, Aspergillus clavatus, Botrytis cinerea, Colletotrichum phomoides, Fusarium oxysporum, Mucor globosus* (No. 77), *Penicillium cyclopium* (No. 110, 121, and 122), *Penicillium oxalicum* (No. 111 and 116), *Penicillium purpurogenum* (No. 113), *Rhizoctonia oslani* (No. 128 and 140), *Rhizopus nigricans* (No. 154 and 160), and *Trichoderma lignorum.*

(d) Each of the molds *Fusarium cephalosporium, Mucor globosus* (No. 83), *Mucor hiemalis* (No. 73 and 74), *Oospora* (No. 94 and 98), and *Penicillium purpurogenum* (No. 119) showed less pectolytic activity at pH 7.0 while the amount of growth decreased. This may indicate that mold growth has an effect on pectolytic activity.

The presence of a partially heat stable factor excreted by *Mucor* is postulated.

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STUDIES ON TENDERNESS OF BEEF. II. VARYING STORAGE TIMES AND CONDITIONS[a, b]

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(Manuscript received July 14, 1955)

Numerous studies have shown that beef becomes first less and then more tender with cold storage between slaughter and consumption. Previous work in this laboratory *(7)* has shown that the rate of heat penetration during cooking has a marked influence on tenderness shortly after slaughter. It seemed advisable to study the effects of somewhat longer storage, and to investigate any alterations in the tenderness pattern which might be occasioned by the handling of the muscles when samples were removed from the carcass.

The experiment was designed also to furnish information on variations in tenderness between steaks cut from the same muscle, since Harrison *et al. (3)*, Ramsbottom, Strandine, and Koonz *(10)*, and Hankins and Hiner *(2)* have all reported variations in tenderness among different portions of the *longissimus dorsi*. This same finding has been reported for pork by Weir *(11)* and Mackay and Oliver *(6)*. Freezing has been reported to have a tenderizing action on beef by some workers *(2, 4)*, but not by others *(1, 8, 9)*. Therefore, a series to compare cold storage with freezing was included.

PROCEDURE

The muscles tested included the *longissimus dorsi* from the 6 animals described in the first paper of this series *(7)*. In addition the *longissimus dorsi* muscles from 2 commercial grade cows were used for the work on effect of freezing.

The muscles were held for different times under various storage conditions as outlined in Table 1. The muscles held "off carcass" were removed from the carcass at the time of slaughter, wrapped loosely, and placed in cold storage. Since it was suggested that the handling and cutting incidental to the removal of the muscle from the carcass might alter the pattern of tenderness change, a comparison was included (Animals IV and V) of muscles ripened off the carcass with the opposite side ripened on the carcass (carcass being intact in cold storage, muscle removed just before cooking). It was postulated that the decided difference in tenderness between these pairs might have been caused by the different rates of cooling of the separate muscle and the intact carcass. Therefore, in the next comparison, one muscle was left on the carcass for the entire storage time, the other one left on long enough for the carcass temperature to drop to that of the storage room, then the muscle removed and stored separately (Animal VI). After storage as indicated, these muscles were cut into one-inch steaks for cooking.

The muscles from Animals VII and VIII were used to check the effect of freezing and different methods of thawing on the pattern of tenderness changes. In addition,

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[b] Presented at the Fifteenth Annual Meeting of the Institute of Food Technologists, Columbus, Ohio, June 13, 1955.

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TABLE 1

Storage times and conditions for longissimus dorsi muscles

Animal	Grade	Side	Storage at 5-7°C.	
I	Good	left	7 days	off carcass
		right	2 days	off carcass
II	Good	left	7¼ days	off carcass
		right	2¼ days	off carcass
III	Prime	left	2¼ days	off carcass
		right	7 days	off carcass
IV	Prime	left	9 days	off carcass
		right	9 days	on carcass
V	Commercial	left	9 days	on carcass
		right	9 days	off carcass
VI	Commercial	left	9 days	on carcass
		right	27 hours on carcass plus 189 hours off carcass (total of nine days)	
VII	Commercial	left	3 days	on carcass
		right	3 days	off carcass
VIII	Commercial	left	3 days on carcass, muscle cut[1]	
		right	3 days on carcass, no cuts	

[1] Cuts made before steak No. 1, between 6 and 7, between 12 and 13, between 18 and 19, and after 24.

the muscles from Animal VIII were used to check the effect on changes in tenderness of cutting the muscle without removing it from the carcass. At the end of the storage indicated in Table 1, the muscles from Animals VII and VIII were cut into steaks, the steaks divided into groups of four, and each set of four assigned to one of the 6 treatments outlined in Table 2. Since the previous work on the first 6 animals for this paper had indicated the possibility of significant tenderness differences between the anterior and posterior portions of a single muscle, each set of 4 steaks included two from the anterior and two from the posterior. Aside from this restriction, the steaks were assigned at random to the different treatments.

The steaks from all the animals were cooked in deep fat at 147°C. to an internal temperature of 63°C. Data on cooking times and losses were recorded. Tenderness was

TABLE 2

Additional storage for steaks from Animals VII and VIII

Group	Storage
1	Cooked immediately after cutting
2	Cut, held at 5-7°C. one day
3	Cut, held at 5-7°C. two days
4	Frozen at −18°C. for 1 day, thawed 1 day at 5-7°C.
5	Frozen at −18°C. for 1 day, thawed in cold running water (½ to 1 hour)
6	Frozen at −18°C. for 1 day, cooked without thawing (thermometers inserted before steaks were frozen)

measured by shearing seven ½-inch cores from each steak on the Warner-Bratzler shearing machine.

The pH of the raw meat was determined for every fourth steak in each muscle. A 5 g. sample was finely minced, mixed with 45 ml. distilled water, and stirred occasionally for 30 minutes. The liquid was decanted, and the pH of the liquid determined with a Beckman pH meter.

RESULTS AND DISCUSSION

pH. The only significant differences found in pH were between the different animals used. The average values were: Animal I, 5.67; II, 5.80; III, 5.47; IV, 5.55; V, 5.38; VI, 5.50; VII, 5.35; VIII, 5.22. Differences due to length of storage, handling, or position were not significant. Apparently, 2 days of cold storage was sufficient to complete the initial drop in pH usually observed in beef after slaughter, and nine days was not long

TABLE 3

Average cooking time and losses by position and by treatment for first six animals

Position	Time (minutes)	Loss (per cent)	Animal	Storage	Time (minutes)	Loss (per cent)
1 (anterior)	4.41	20.0	I	7 days off carcass	5.35	23.1
2	4.85	19.9		2 days off carcass	5.73	23.6
3	5.22	21.1	II	7¼ days off carcass	5.60	22.4
4	5.00	22.1		2¼ days off carcass	6.13	22.1
5	4.93	17.7	III	2¼ days off carcass	4.85	20.6
6	5.60	21.9		7 days off carcass	6.42	24.2
7	5.45	19.7	IV	9 days off carcass	6.70	20.1
8	5.60	20.2		9 days on carcass	6.17	21.9
9	6.06	21.7	V	9 days on carcass	6.10	24.8
10	5.89	21.5		9 days off carcass	6.16	21.2
11	6.37	23.2	VI	9 days on carcass	5.17	19.0
12	5.52	21.3		27 hours on, 189		
13	5.52	21.6		off carcass	5.54	22.0
14	5.69	21.6				
15	6.06	22.6				
16	6.59	24.3				
17	6.75	25.8				
18	6.88	24.7				
19	6.63	25.1				
20	5.97	24.1				
21	6.67	26.0				
22	6.48	25.2				
23	5.72	24.3				
24 (posterior)	5.84	23.3				
Grand average	5.83	22.4				
Standard error	0.08	0.24				
Correlation between time and loss, rxy = 0.595**						
F-values, analysis of variance position	1.18	2.4**		treatment	5.49**	4.35**
anterior vs. posterior	65.82**	65.1**				

enough to occasion the slight rise in pH with longer storage observed by Wierbicki *et al. (12)*.

Cooking time and losses. The average cooking times and losses for the muscles from the first 6 animals are listed in Table 3 by position of the steak in the muscle, and by treatment of the muscle. Much of the variation due to position was attributable to the differences between anterior and posterior portion of the muscle. Both cooking time and losses also varied appreciably according to the storage treatment received. However, it does not seem advisable to place too much emphasis on these data, as small variations in thickness of steak and/or in placement of the thermometer could cause significant variations in time in a cooking method as rapid as the one used. Also, the highly significant correlation between cooking loss and time indicates that a considerable portion of the differences in cooking loss was due to variations in the cooking time.

The average cooking times and losses for Animals VII and VIII are listed in Table 4. The major differences found here were due to the treatment of the steaks. The steaks which had been frozen required more time to cook and had greater cooking losses, especially in the steaks which were cooked without previous defrosting. Again, the high correlation between time and loss indicated that the cooking losses varied with the cooking time.

TABLE 4

Average cooking times and losses by storage of muscle and by treatment of steak, for Animals VII and VIII

Animal	Time min.	Loss %		Time min.	Loss %
VII 3 days on carcass	8.62	30.29	cooked immediately	7.66	26.57
3 days off carcass	9.16	28.74	one day additional cold storage	7.06	25.65
			two days additional cold storage	7.52	26.16
VIII 3 days on carcass, cut	8.66	28.35	frozen, thawed 1 day at 5-7°C.	8.81	28.56
3 days on carcass, whole	8.66	27.13	frozen, thawed in water	7.82	31.66
			frozen, cooked without thawing	13.69	33.78
F-values, analysis of variance	< 1	2.40		24.40**	8.04**
Correlation between cooking time and loss, rxy = 0.76**					

Tenderness. The average shear values and F-values for the steaks from the first 6 animals are listed in Table 5, and for those from Animals VII and VIII in Table 6. In most cases, grade comparisons are complicated by differing treatments. However, grade comparisons are possible between Animals II *(good)* and III *(prime)*, and between Animals IV *(prime)* and V *(commercial)*. In each case, the *prime* animal gave more tender steaks than the *good* or *commercial*, although the difference was small between *prime* and *good* after 7 days of storage.

Treatment of Muscle. In the animals where the paired muscles had different cold storage times (I, II, III) longer storage resulted in more

TABLE 5

Average shear values for steaks from first six animals

Position	Shear (pounds)	Treatment		Shear (pounds)
		Animal	Storage	
1 (anterior)	7.22	I	7 days off carcass	7.97
2	6.73		2 days off carcass	11.28
3	6.41	II	7¼ days off carcass	5.86
4	7.49		2¼ days off carcass	10.28
5	6.68	III	2¼ days off carcass	7.51
6	7.20		7 days off carcass	5.44
7	7.20	IV	9 days off carcass	6.98
8	7.55		9 days on carcass	4.70
9	8.05	V	9 days on carcass	6.60
10	7.61		9 days off carcass	10.45
11	7.64	VI	9 days on carcass	6.25
12	7.49		27 hrs. on, 189 hrs. off carcass	8.08
13	7.23			
14	7.56			
15	7.72			
16	7.95			
17	9.24			
18	8.39			
19	7.45			
20	8.50			
21	8.49			
22	8.88			
23	7.44			
24 (posterior)	7.78			
F-values, analysis of variance				
Position	1.68*	Treatment		44.99**
anterior vs. posterior	22.13**			

TABLE 6

Average shear values for steaks from Animals VII and VIII

Treatment of muscle		Shear (pounds)	Treatment of steak	Shear (pounds)
Animal	Storage			
VII	3 days on carcass	10.63	cooked immediately	12.33
	3 days off carcass	12.85	1 day additional cold storage	11.33
			2 days additional cold storage	10.69
VIII	3 days on carcass, cut	11.80	frozen, thawed 1 day at 5-7°C.	10.08
	3 days on carcass, not cut	9.60	frozen, thawed in water	11.11
			frozen, cooked without thawing	11.79
F-values, analysis of variance				
muscle treatment		28.32**	Steak treatment	5.98**

tender steaks in every case. This was true for all but two of the 71 pairs of individual steaks, as well as for the averages.

In the remainder of the animals, each muscle was stored for the same length of time as its pair mate, but one of each pair was handled in some way before or during the storage period—removal from the carcass before

TABLE 7

F-values for significance of differences in shear within each muscle, and average and LSD for each muscle (calculated from 7 individual shear readings for each steak)

Animal	Side	F-values		Average shear		Entire muscle		
		Between anterior and posterior	Between steaks	Anterior	Posterior	Average shear	LSD[1] 05	LSD[2] 01
I	left	38.11**	1.78	7.10	8.83	7.97	1.91	2.53
	right	< 1	5.16**	11.35	11.20	11.28	2.31	3.06
II	left	6.07*	2.00*	5.62	6.11	5.86	1.36	1.79
	right	7.74**	4.41**	9.79	10.80	10.29	2.48	3.28
III	left	< 1	3.86**	7.54	7.48	7.51	1.31	1.73
	right	11.26**	2.44**	5.09	5.76	5.44	1.33	1.76
IV	left	6.09**	2.75**	6.65	7.31	6.98	1.84	2.43
	right	4.26*	1.22	4.85	4.55	4.70	1.00	1.32
V	left	< 1	2.30**	6.70	6.52	6.60	1.73	2.29
	right	96.09**	6.34**	8.38	12.52	10.45	2.89	3.82
VI	left	< 1	3.84**	6.18	6.31	6.24	1.49	1.96
	right	44.30**	6.95**	6.89	9.28	8.08	2.46	3.25

[1] LSD_{05}—Least significant difference from the mean, $p \leq 0.05$.
[2] LSD_{01}—Least significant difference from the mean, $p \leq 0.01$.

cooling (IV, V, VII), removal after the carcass had cooled (VI), or cutting through the muscle without removal from the carcass (VIII). Any type of handling apparently interfered with the tenderizing process, as shown by the averages. This was also true in 102 of the 120 individual pairs of steaks. In the 18 exceptions, the steak which had been handled had only very slightly lower shear readings than its pair mate. The more rapid rate of cooling in the excised muscle was not the only factor governing the alteration in development of tenderness, as the muscle cooled on the carcass then removed for additional storage was not as tender as that stored on the carcass. Cutting the muscle on the carcass also interfered with the development of the tenderness. This parallels the findings of Koonz, Darrow, and Essary *(5)* on poultry.

Treatment of Steaks. Additional treatment of the steaks (either cold storage or freezing and thawing) increased their tenderness. However, when the length of storage time was the same, cold storage was nearly as effective in tenderizing as was freezing. Longer frozen storage might well alter this result. The frozen steaks cooked without thawing were less tender than those thawed in water or in the refrigerator.

Position. Data from the first 6 animals indicated a highly significant difference in tenderness between the anterior (first 12 steaks) and posterior portions of the muscle, and a significant difference within each of these portions. To clarify this finding, a more detailed analysis was made of these data. The results of this analysis are shown in Table 7. The anterior was more tender than the posterior in 7 muscles, the posterior more tender in one, and in four there was no significant difference between the 2 halves. The individual steaks varied considerably in tenderness, even after the anterior-posterior effect was removed. In order to locate the significantly different steaks, the average shear and least significant difference at $p < 0.05$, and $p < 0.01$ were calculated for each muscle. Then, the average shear for each steak was compared with the average shear value for the muscle. The steaks which differed significantly from the muscle average are indicated in Table 8. It is apparent that both grade and treatment are important in determining the uniformity of the muscle with respect to shear tenderness. The muscles from the *prime* were more nearly uniform than those from the *good* animals, and the muscles from the *good* grade more uniform than those from the *commercial* animals. Increasing storage tended to increase uniformity, while handling the muscles before or during storage decreased the uniformity.

SUMMARY AND CONCLUSIONS

Eight pairs of *longissimus dorsi* muscles from *prime*, *good* and *commercial* grade beef animals were used to study the effects of various cold storage and freezing and thawing treatments on cooking time, cooking losses, and shear tenderness of steaks fried in deep fat.

Significant differences in cooking time and losses were found among the various treatments. The steaks which had been frozen required slightly longer to cook and had higher cooking losses than those which had not been frozen. The frozen steaks cooked without thawing had the highest cooking time and losses. Steaks from the anterior portion of the muscle

TABLE 8

Steaks varying significantly from average shear for muscle

Animal	I		II		III		IV		V		VI	
Grade	Good		Good		Prime		Prime		Commercial		Commercial	
Storage / Position	7 days off	2 days off	7¼ days off	2¼ days off	2¼ days off	7 days off	9 days off	9 days on	9 days on	9 days off	9 days on	27 hrs. on, 189 hrs. off
1 (anterior)		−				(1)			(1)			−
2										−		−
3	−	− −										−
4									(1)	−		−
5		+ +								−		
6										− −		
7		+										
8						−						
9					+						+ +	
10												
11												
12												
13												
14							−		+			
15	+											
16									+			
17	+		+	+ +						+ +	+ +	
18				+ +								
19												
20		+										+ +
21		−			+					+ +		
22										+ +		+ +
23							+ +					
24					− −	+ +						+ +

(1) missing data

	$p < 0.05$	$p < 0.01$
Less tender than average	+	+ +
More tender than average	−	− −

tended to cook more rapidly and have lower cooking losses than those from the posterior portion. The high correlation between cooking time and losses indicated that one of the major factors affecting cooking loss was the time required to cook the steak.

The *prime* grade steaks were more tender than those from the *good* or *commercial* grades, although increase of cold storage tended to minimize the grade difference.

As expected, increased length of cold storage increased the tenderness of the steaks. Any handling of the muscle, such as removal from the carcass before or during storage, or cutting of the muscle on the carcass, interfered with the tenderizing process, and resulted in less tender steaks than those aged in the normal manner on the carcass.

Additional cold storage or freezing of the steaks after 3 days aging on the carcass increased the tenderness. Within the time studied (0, 1, or 2 days additional storage) cold storage was nearly as effective as freezing and thawing in increasing the tenderness. Frozen steaks cooked without thawing were less tender than those thawed before cooking.

In most of the muscles, the anterior and posterior portions differed in tenderness, the anterior being the more tender in the majority of cases. There was also considerable variation in individual steaks within each portion, suggesting the need for careful consideration in selecting samples for comparability when planning experiments. Normal (on carcass) cold storage of 7 to 9 days after slaughter tended to minimize these variations. Also, the variations were more pronounced in beef of lower grade.

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STUDIES ON TENDERNESS OF BEEF. III. SIZE OF SHEAR CORES: END TO END VARIATION IN THE *SEMIMEMBRANOSUS* AND *ADDUCTOR*[a]

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The Warner-Bratzler shearing machine has been used by many investigators to measure objectively the tenderness of meat. Results obtained, when compared to judging panel scores, have ranged from very high *(3, 4)* to very low *(1)* correlation. Hurwicz and Tischer *(2)* found that the slope of the line formed by plotting shear force versus time was more nearly constant than was the shear force itself.

Most of the work with this instrument has been done by shearing one-inch cores of meat. However, Ramsbottom and Strandine *(4)* have reported high correlation between panel scores and shears for one-half inch cores. One-half inch cores are easier to cut without including heavy deposits of connective tissue and fat. Also, more one-half inch cores can be cut from a single steak, giving more readings per steak. Therefore, it seemed of interest to run a series of direct comparisons between shears of one-half and one-inch cores. At the same time it was possible to collect data on the effect of cold storage on the shear tenderness of the *semimembranosus* and *adductor* muscles of the beef round, and on the end to end variations within these muscles.

METHODS

The *semimembranosus* and *adductor* muscles from two good grade and one prime grade beef animals were used. These were the same animals as the first three listed in a previous paper *(5)*. The muscles were removed from the carcass at the time of slaughter. The muscles from one side of each animal were held in cold storage (5-7°C.) 6 days, those from the other side for 8 or 9 days. After storage, each muscle was cut into one-inch steaks. The *semimembranosus* yielded ten steaks, the *adductor* five. The steaks were cooked in deep fat at 147°C. to an internal temperature of 63°C. Cooking times and losses were recorded. The cores for shearing were cut in pairs—one of one-inch, the other of one-half inch diameter. As many pairs were cut from each steak as could be obtained without utilizing the edge or any areas showing obvious streaks of connective tissue and/or fat. Samples of the raw steaks were utilized for pH determinations using the method described in paper II of this series *(5)*.

RESULTS AND DISCUSSION

There was a highly significant difference in pH of the 3 animals. The average pH for Animal I was 5.50, for Animal II, 5.67, and for Animal III, 5.43. These were in the same order as previously reported for the *longis-*

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TABLE 1

Average cooking times and losses for steaks from the semimembranosus and adductor muscles

	Cooking time (minutes)			Cooking losses (per cent)		
	All steaks	*Semimembranosus*	*Adductor*	All steaks	*Semimembranosus*	*Adductor*
Treatment						
Animal I, good grade						
6 day storage	5.88	5.93	5.76	27.67	26.94	29.12
9 day storage	5.31	5.13	5.65	27.73	27.44	28.30
Animal II, good grade						
6 day storage	7.12	6.64	8.07	27.63	26.64	29.60
9¼ day storage	6.05	6.28	5.58	28.47	28.41	28.58
Animal III, prime grade						
6 day storage	6.95	7.01	6.87	26.54	23.66	30.58
8 day storage	5.21	5.26	5.11	24.97	23.91	27.10
F-values, variance						
Muscle	<1			9.51**		
Treatment	4.85**			1.65		
Position						
1 (anterior)		7.61	5.96		30.77	29.32
2		6.21	6.05		24.18	27.27
3		5.92	6.07		26.70	27.85
4		5.03	6.68		25.45	30.27
5 (posterior Ad)		6.49	6.11		27.28	29.70
6		5.56			25.32	
7		5.00			24.38	
8		5.40			24.10	
9		6.65			27.16	
10 (posterior SM)		6.10			27.64	
F-values, variance						
Position		2.26*	<1		1.80	1.11
Treatment		3.00*	3.26*		2.46*	<1

* $p < 0.05$
** $p < 0.01$

simus dorsi (5). The pH values for the muscles and storage times did not differ significantly.

Cooking time and losses. The average cooking times and cooking losses are listed in Table 1. When the data for all the steaks were considered together, the principal differences in cooking time were due to the various treatments, and to the animals used, as the time decreased somewhat with increasing storage, and the steaks from Animal II required the longest while those from Animal I took the shortest time. The chief source of variation in cooking losses was the difference between the 2 muscles, the *adductor* steaks having greater losses than the *semimbranosus* steaks. The various treatments did not affect the losses as they did the time, although the correlation between time and losses ($r_{xy} = 0.619^{**}$) indicated that increased cooking time tended to increase the cooking losses.

When the muscles were considered separately, increasing storage tended to decrease the cooking time, but to increase the cooking losses for the *semimembranosus* steaks. In the *adductor*, the cooking time also decreased slightly with increased storage, while the cooking losses did not differ significantly.

Shear. *Size of Core.* While there was not complete agreement in the trend of values for the one-half and one-inch cores, the high correlation between the two ($^{r}xy = 0.748^{**}$) and the similarity in significance of F-values from the analysis of variance suggest that either size core may be used for determination of shear tenderness (Table 2).

TABLE 2

Shear force values for ½-inch and 1-inch cores from steaks from the semimembranosus and adductor muscles

Factor	½-inch cores, shear (lbs.)			1-inch cores, shear (lbs.)		
	All steaks	*Semimembranosus*	*Adductor*	All steaks	*Semimembranosus*	*Adductor*
Treatment						
Animal I, good grade						
6 day storage	9.68	11.03	7.00	23.71	26.51	18.10
9 day storage	8.94	9.30	8.23	21.15	22.98	17.48
Animal II, good grade						
6 day storage	7.43	7.96	6.36	19.49	20.82	16.83
9¼ day storage	7.03	7.33	6.43	18.45	19.22	16.91
Animal III, prime grade						
6 day storage	6.91	7.06	6.70	18.15	19.29	16.56
8 day storage	7.33	7.93	6.14	19.84	21.37	16.78
F-values, variance						
Muscle	31.90**			37.72**		
Treatment	10.72**			5.23**		
Position						
1 (anterior)		6.86	6.53		15.97	15.55
2		7.86	6.55		18.43	18.37
3		7.82	7.07		20.71	18.07
4		8.97	7.34		20.85	17.77
5 (posterior Ad)		8.45	6.56		23.05	15.79
6		8.95			23.88	
7		9.67			24.74	
8		9.99			25.31	
9		8.59			25.57	
10 (posterior SM)		8.12			20.81	
F-values, variance						
Position		5.17**	< 1		12.45**	1.74
Treatment		22.11**	1.82		15.73**	< 1

Treatment. The differences between treatments were highly significant, principally due to treatment effects on the steaks from the *semimembranosus* muscle. In these steaks, within the *good* grade, the first animal was less tender than the second, while the second *good* and the *prime* grade were very similar. The steaks from the *prime* animal became less, rather than more, tender with longer storage. On the other hand, the steaks from the *adductor* muscles did not differ significantly in shear with animal, grade, or length of storage.

Position. The *adductor* muscle was quite uniform throughout in shear tenderness, while the *semimembranosus* showed highly significant differences due to position. Calculation of the least significant differences from the mean values for one-half and one-inch cores for the *semimembranosus* steaks gave the following results:

	one-half inch cores	*one-inch cores*
Mean ± LSD_{05}	8.51 ± 1.13	21.83 ± 2.48
Mean ± LSD_{01}	8.51 ± 1.52	21.83 ± 3.32
Shear values < mean − LSD_{01}	steak No. 1	steaks No. 1, 2
Shear values > mean + LSD_{05}	steaks No. 7, 8	steak No. 7
Shear values > mean + LSD_{01}		steaks No. 8, 9

The data suggest that the section of the *semimembranosus* from steak No. 3 through steak No. 6 was reasonably uniform with respect to shear.

SUMMARY AND CONCLUSIONS

Steaks from the *semimembranosus* and *adductor* muscles of two good grade and one prime grade beef carcasses were used to study the effects of storage, muscles, and position within the muscle on cooking time, cooking losses, and shear tenderness. A comparison was made of the use of one-half inch and one-inch cores for the determination of shear force.

The cooking time varied chiefly among animals, and decreased somewhat with increasing storage. The major variation in cooking losses was due to muscle, the steaks from the *adductor* having higher losses than those from the *semimembranosus*. The highly significant positive correlation between cooking time and loss indicated that the losses tended to increase with increased cooking time.

The close agreement in trend between shears from one-half and one-inch cores suggests that either size may be used to measure shear tenderness. The *adductor* muscle proved to be quite uniform in shear tenderness, regardless of grade, treatment, or position of the steak in the muscle. On the other hand, the shear tenderness of steaks from the *semimembranosus* muscles was affected by animal, grade, storage, and position. The steaks from the *good* grade animals became more tender between 6 and 9 days of storage, while those from the *prime* aimal were less tender after 8 days' storage than after 6. The first and second steaks from the anterior portion of the muscle were more tender than the center portion, while the seventh, eighth, and ninth steak from the posterior portion were less tender. The four-inch block of steaks three through six tended to be quite uniform in shear tenderness.

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SORBIC ACID AS A FUNGISTATIC AGENT FOR FOODS. IX. PHYSICO-CHEMICAL CONSIDERATIONS IN USING SORBIC ACID TO PROTECT FOODS

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In preceding papers it was shown that sorbic acid is an effective non-toxic and practical fungistatic agent for foods. Indeed it is unique among food additives, being a metabolizable food *(3, 4)* added for protective purposes to another food. The published reports have dealt with the use of sorbic acid to inhibit mold spoilage in cheese. For a review of the earlier developments in four different laboratories, reference is made to the Smith and Rollin summary paper *(14)*.

EXPERIMENTAL PART

In investigating the application of sorbic acid to other food products, a number of physio-chemical studies were carried out to obtain basic information of value in supplementing foods with this fungistatic agent. Earlier studies in this field have dealt with the spectrophotometric determination of sorbic acid in foods *(5, 6)* and on wrappers *(6)*, the degrees of resistance to oxidative deterioration of sorbic acid as such, on wrappers, and in foods *(8)*, and the importance of maintaining an adequately high ratio of sorbic acid to mold concentration *(9)*. Additional basic information, accumulated since the earlier investigations, is now being reported.

Purity of Commercially Available Sorbic Acid

Studies were conducted on ten lots of commercially available sorbic acid[a] to determine the purity of the material available to investigators for use in both laboratory and plant studies. Most analyses were conducted on the materials as received[b] but with results expressed on a moisture-free basis.

Melting point values were obtained on the sorbic acid samples after drying for 4 hours at 50°C. in a vacuum oven at 20 mm. (Hg) pressure. In determining equivalent weight the sorbic acid was dissolved in neutral isopropanol and then titrated with 0.1 N sodium hydroxide solution to the first end-point with phenolphthalein as the indicator. Sorbic acid concentrations were calculated from the found equivalent weights using 112.12 as the theoretical for pure moisture-free sorbic acid. The ultra-violet

[a] These samples were selected at random from aliquots of production lots retained by Carbide and Carbon Chemicals Company, New York.

[b] Sorbic acid loses moisture very rapidly during weighings at room temperature. For this reason, glass stoppered weighing bottles were employed in taking samples for analyses.

absorption tests were conducted on isopropanol solutions[c] of the sorbic acid samples, and extinction ratios calculated to define the shape of the absorption curves. The color intensities of the commercially available sorbic acid samples were determined spectrophotometrically on isopropanol solutions. The readings in both the ultraviolet and visible regions of the spectrum were made with the Beckman Spectrophotometer. Ashings of the samples were carried out in platinum dishes. Heavy metal contents were determined according to a spectrophotometric adaptation of the U.S.P. method for benzoic acid *(15)*. Moisture analyses of the test samples were conducted according to the toluol distillation procedure *(10)*.

Results of the analyses of the lots of commercially available sorbic acid

TABLE 1

Values obtained in analyzing ten lots of commercially available sorbic acid (s.a.) selected at random

Analysis[1]	Test material	Average value	3σ limits
Melting point	Moisture-free s.a.	134.5°C.	133-136°C.
Equivalent weight	0.5% s.a. in isopropanol	112	110.5-113.5
Sorbic acid content	Calculated value	100%	101.5-98.5%
$E\,\frac{1\%}{1\ cm}$ 254 mμ	0.00025% s.a. in isopropanol	2420	2300-2540
E245 mμ/E254 mμ	0.00025% s.a. in isopropanol	0.83	0.80-0.86
E265 mμ/E254 mμ		0.76	0.73-0.79
Color, light transmission at 400 mμ	3.00% s.a. in isopropanol	92%	86-98%
Ash content	25 gram aliquot of s.a.	0.07%	0.00-0.15%
Heavy metal	4.00% s.a. in acetone	less than 10 p.p.m.	less than 10 p.p.m.
Moisture	10 gram aliquot of s.a.	6.5%	3-10%

[1] All analyses, other than melting point, were conducted on the samples as received. Based upon the moisture figures obtained, all other values were calculated to a dry basis.

are summarized in Table 1. Three sigma limits have been calculated to define the reproducibility of the sorbic acid; 997 batches out of a 1000 might be expected to yield values encompassed by these 3σ limits. The most precise test employed in this study is the titrimetric determination of equivalent weight. The melting point and particularly the spectrophotometric values are important in being more specific for sorbic acid. Based upon the results of these three tests, it has been concluded that the purity of commercially available sorbic acid is $100.0\% \pm 0.5\%$ (one standard deviation), calculated to a moisture-free basis. The other tests in this series are of practical value to users of sorbic acid and the one for heavy metal content has been included to show that the sorbic acid is regularly free of such undesirable contaminants.

[c] Whereas sorbic acid is in aqueous solution in the spectrophotometric determination of the fungistatic acid in foods *(5, 6)*, isopropanol is the preferred solvent in testing crystalline sorbic acid for purity. Errors due to incomplete solubility of the sorbic acid are avoided when isopropanol is employed as the solvent.

Solubility of Sorbic Acid

The solubility of sorbic acid in various solvents is important in determining (a) expeditious ways for adding the fungistatic agent to foods and (b) the maximal concentrations that can be retained in solution. In conducting tests with these objectives in mind, the sorbic acid was added in obvious excess to the test solvent in a closed volumetric flask. The mixture was heated in a hot-water bath to a temperature of 140°F., agitated at this temperature by mild shaking, and allowed to cool slowly in the dark to the desired temperature. After 16 hours at each of the desired temperatures, the undissolved sorbic acid was filtered off, and the clear solutions analyzed spectrophotometrically *(6)* for sorbic acid content.[4] The results in this series of studies of sorbic acid solubility are summarized in Table 2.

TABLE 2

Solubility of sorbic acid in solvents of potential value in supplementing foods with the fungistatic acid

Solvent	Sorbic acid in solution at 74°F.
	gm. per 100 gm.
Ethanol (95.0%)	14.5
Acetic acid (99.8%)	12.3
Lactic acid (85.5%)	2.26
Citric acid (50.0%)	0.26
Phosphoric acid (85.0%)	0.12
Water (distilled); solution at pH 3.1	0.16
Buffered with phosphate to pH 4.4	0.22
to pH 5.9	1.02
Limpid vegetable oil (soybean oil)	0.52

Ethyl alcohol, the vehicular material for many flavoring compounds in foods, is an excellent solvent for sorbic acid. Among the acids found in foods and employed in the present study, acetic acid is by far the best solvent for sorbic acid. The fungistatic agent is soluble in water at room temperature (74°F.) to the extent of 0.16%. In buffered solutions with pH values well above that of 3.1 (that of sorbic acid in distilled water), more of the fungistatic agent can be dissolved. This is due, of course, to partial conversion of sorbic acid to the highly soluble salts of sorbic acid during solution of the acid in the buffered solutions. Sorbic acid is about three times as soluble in edible oils and fats as in water.

In Figure 1 are plotted curves showing the extent to which sorbic acid solubility in water can be increased with increasing temperature, or with increasing concentration of ethanol or acetic acid in the water. Information supplied by Table 2 and Figure 1 should be of value in determining expeditious ways for adding sorbic acid to food formulations.

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[4] In these and in subsequent spectrophotometric analyses of test systems for sorbic acid content, absorbancy readings from 245 to 270 millimicrons were taken. In every case the absorption curve was typical of that of pure sorbic acid, with the position of the absorption maximum dependent upon the pH of the test solution *(5, 6)*.

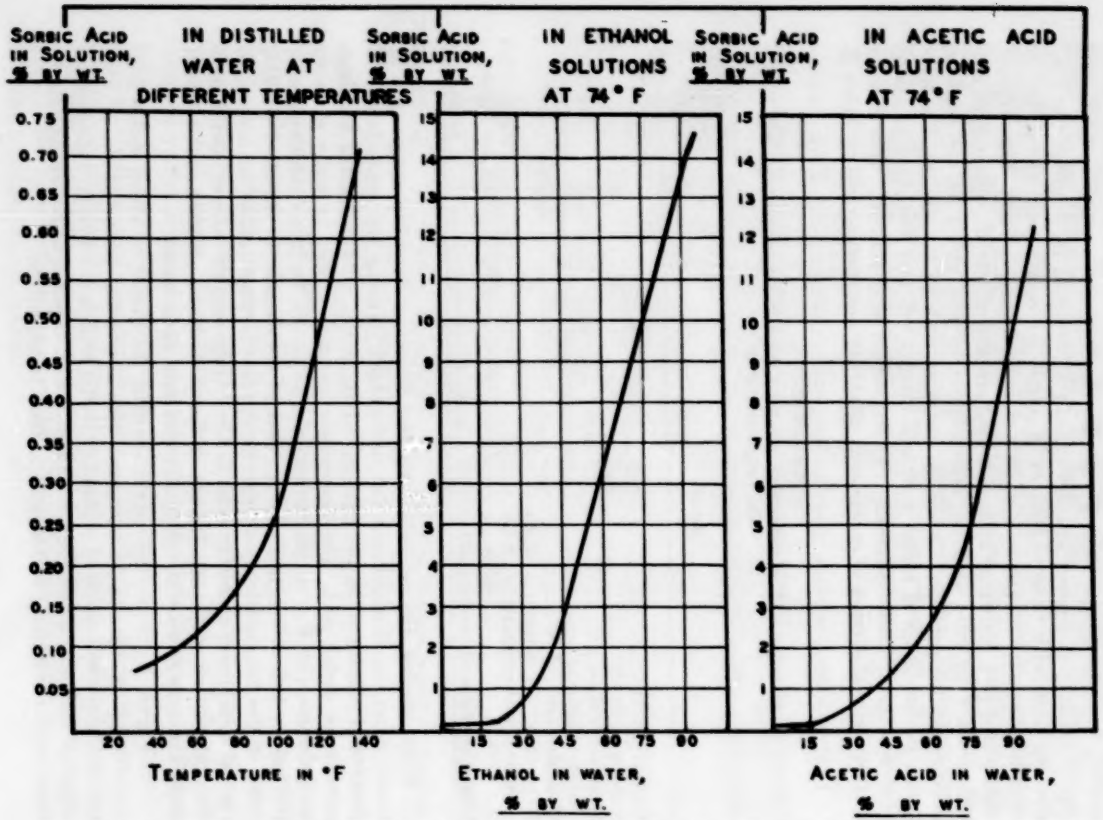

Figure 1. Increase in solubility of sorbic acid in water with increasing temperature, or with increasing concentration of ethanol or acetic acid in water.

Phase Distribution Studies with Sorbic Acid

A series of test solutions, containing sorbic acid in water with other solutes (the latter as single concomitant additives or in combinations), were prepared using the same procedure described in the preceding section to effect maximal solubility of the sorbic acid. To each filtrate was added an equal part by weight of limpid vegetable oil (soybean oil), the mixture shaken mechanically for 30 minutes and then held in the dark for 16 hours at room temperature (74°F.). The aqueous phases before and after extraction with the vegetable oil were analyzed spectrophotometrically *(6)* for sorbic acid content. The results of this study on the influence of (a) other soluble food constituents and (b) the concomitant presence of an edible fat phase on the solubility of sorbic acid in the aqueous phase are plotted in Figure 2.

The distribution ratio reflecting the solubility of sorbic acid in fat to that in water is 3.0. The presence of soluble food components, such as glucose, sucrose or sodium chloride, at high concentrations reduces the solubility of the sorbic acid in water. Phase distribution studies on such

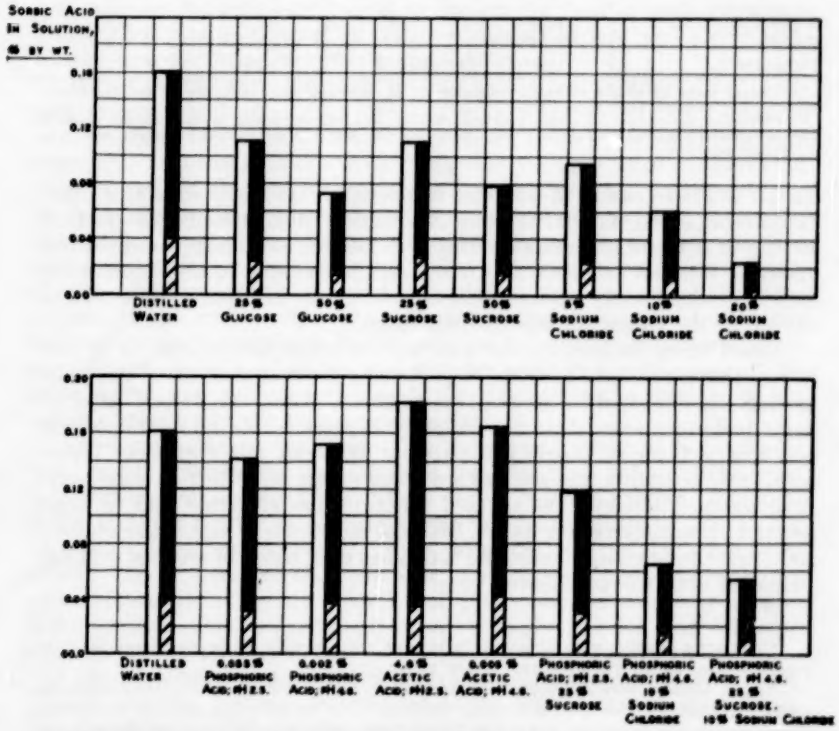

Figure 2. Solubility at room temperature (74°F.) of sorbic acid (a) in the specified aqueous test system, □; (b) in the aqueous phase after equilibration with an equal part by weight of a limpid vegetable oil, ▨; and (c) in the vegetable oil extract, ■.

test systems indicate that the ratio of sorbic acid solubility in oil to water of 3.0 is increased to values as high as 5.0 as a result of the concomitant presence of other water-soluble components in the aqueous phase. However, the acids in concentrations, as found in foods, modify to an insignificant degree the solubility of sorbic acid in the aqueous phase both before and after equilibration with the vegetable oil phase.[e] The influences of the solutes when added in combinations on sorbic acid solubility in water are not additive. Sorbic acid solubility under such circumstances is the same as that for the system containing the one solute which affected sorbic acid solubility to the greatest degree.

In Table 3 are shown the results obtained in comparable phase distri-

TABLE 3

Phase distribution studies[1] with fungistatic acids

Fungistatic acid	Concentration in			Distribution ratio, b/a
	Original aqueous phase	Extracted aqueous phase (a)	Vegetable oil phase (b)	
Sorbic	0.160	0.040	0.120	3.0
Benzoic	0.320	0.045	0.275	6.1
Propionic	0.280[2]	0.240	0.040	0.17

[1] Equal parts by weight of limpid vegetable oil and distilled water solutions of the fungistatic acids shaken together (system at 74°F.).

[2] Whereas the values for sorbic acid and benzoic acid concentrations in the original aqueous phases represent maximal solubilities, this value for the highly water-soluble propionic acid was arbitrarily selected.

bution studies conducted at room temperature (74°F.) with two other fungistatic acids. The latter were determined titrimetrically, the method having in it blank titrations conducted on the control unsupplemented test systems. Whereas propionic acid is predominantly water-soluble and benzoic acid is predominantly fat-soluble, sorbic acid falls in between these two as shown by the values for distribution ratio.

Based upon findings in our own and in other laboratories, it is well known that sorbic acid activity, in protecting foods against mold and yeast spoilage, is increased in an acid environment. Thus, the overall effect of acids including even those that slightly depress sorbic acid solubility in water is in favor of fungistatic activity. Whereas, salt noticeably lowers sorbic acid solubility, the salt has a marked synergistic effect on sorbic acid fungistasis. Therefore, the reduced concentration of sorbic acid in brine solutions is no deterrent to its use. Likewise in systems of high sugar content, sorbic acid fungistasis is increased[f]; this has been noted in systems with pH values of even 6.5 and above.

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[e] The same results were obtained in similar studies with citric and lactic acid in concentrations of 1.0 per cent and less in water.

[f] In an aqueous solution, high in sugar content, sorbic acid is an effective fungistatic agent provided the test system remains uniform. Due to refluxing activity within the closed unmolested container, a surface film of pure water may condense on the walls of the container and on the syrup itself. On such surfaces mold growth can occur since sorbic acid migration to these vulnerable areas may not be sufficiently rapid. Mixing the contents of the container prevents this sequence of events.

Fat in the food lowers the concentration of sorbic acid in the aqueous phase—the phase which must be protected in order to prevent mold growth. But even here sorbic acid enjoys a much more favorable distribution ratio than benzoic acid. When tested by the agar-disk-plate method, sorbic acid is at least three times as effective as benzoic acid *(11)*. Similar results have been obtained in comparing the relative effectiveness of the two fungistatic agents in protecting margarine *(12)*, cheese *(13)*, fish *(2)*, bread *(1)*, and cake *(1)* products. The superiority of sorbic acid over propionic acid is independent of distribution ratio, since in this property propionic acid has the advantage. Sorbic acid has been found to be about four times as effective as propionates in protecting cheese *(13)*, bread *(1)*, and cake *(1)* products.

Sublimation of Sorbic Acid

Sorbic acid on a food wrapper is not sufficiently volatile under ordinary circumstances to cross air-pockets between wrapper and food. The fungistatic acid is capable of migrating from wrapper into food in direct contact with it *(7)* and in this way eventually reaching the surface of the food separated by air pocket from the wrapper. Because migration of sorbic acid may not be sufficiently rapid to protect the food at such vulnerable areas, it has been recommended that close contact of food and sorbic acid–treated wrapper be an objective in packaging *(13)*.

Recent studies have demonstrated that sorbic acid sublimes from wrappers so readily at elevated temperatures that air pockets in packaging need no longer be a critical factor in packaging foods in sorbic acid–treated wrappers. In these studies Payne Permeability Cups[5] were employed. A piece of each of the test wrappers was inserted between the two sections of the cup with the sorbic acid–coated surface of the wrapper facing the cup cavity. Each of the clamped assemblies on an asbestos mat was then placed in a large thermostatically controlled electric oven for various periods of time. Sublimed sorbic acid was deposited as fine crystals on the inner surface of the cup cavity. The sublimed material was dissolved, after cooling to room temperature, in 95% ethanol for spectrophotometric determination of quantity of sorbic acid and for positive identification of the sublimed material as pure sorbic acid *(6)*. Typical results obtained in the course of this study are summarized in Table 4.

Based upon the results obtained in the present study, it is concluded that, among those tested cellophane is the preferred wrapping material from which sorbic acid may be sublimed during the flash heating. Exposure of the cellulose acetate or glassine sheets to a temperature of 550°F. for 30 seconds is so severe that some loss of sorbic acid to the atmosphere occurs. This is apparent from the odor of sorbic acid in the surrounding air and from the failure to account for all the sorbic acid originally present in testing for sorbic acid retained on the wrapper and for that sublimed into the cup cavity.

Films of pliofilm and polyethylene are unsatisfactory as the base sheet from which sorbic acid may be sublimed by the flash heating process. These films decompose at the elevated temperatures.

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[5] Obtained from the Fisher Scientific Company, New York.

TABLE 4

Heat sublimation of sorbic acid from wrappers

Wrapper[1]	Exposure		Amount of sorbic acid			
	Time	Temp.	Unheated disc	Sublimed	Retained	Recovered
	sec.	*°F.*		*mg. per sq. in.*		
Sorbic acid on cellophane (MSAT 86); binder, 6% by wt. of the sorbic acid.	30	350		0.28	2.07	2.35
	30	450	Avg.	1.37	0.78	2.15
	7	550	2.20	1.45	0.90	2.35
	30	550		1.65	0.37	2.02
Sorbic acid on cellulose acetate sheet of 2 mil thickness ("Lumarith"); binder, 6% by wt. of the sorbic acid.	30	350		0.48	3.90	4.38
	30	450	Avg.	1.64	2.33	3.97
	7	550	4.10	1.42	2.48	3.90
	30	550		1.77	0.79	2.56
Sorbic acid on glassine (25 lb. per ream); binder, 6% by wt. of the sorbic acid.	30	350		0.85	2.95	3.80
	30	450	Avg.	2.05	1.15	3.20
	7	550	4.10	2.53	2.02	4.55
	30	550		2.18	0.32	2.50
Sorbic acid on cellophane (MSAT 86); thermoplastic wax coating, 1400% by wt. of the sorbic acid.	30	350		0.14	3.66	3.80
	30	450	Avg.	0.69	2.60	3.29
	7	550	3.80	0.27	3.66	3.93
	30	550		2.65	1.15	3.80

[1] Wrappers kindly furnished by Milprint, Inc., Milwaukee, Wisconsin.

The practical value of the sublimation procedure has been demonstrated in studies on wrapped foods. Heating such a food under preferred conditions, at 450° or 550°F. for 30 or 7 seconds, respectively, results in sublimation of the sorbic acid from the inner surface of the wrapper onto the surface of the food without heating the latter to elevated temperatures. Indeed, the food supplies a cool condensing surface for the volatilized sorbic acid so that reverse sublimation to the wrapper does not occur. Tests conducted on a variety of foods, such as luncheon meats, fresh meats, processed and natural cheeses (including Swiss cheese with eyes), fruits, cakes, and incompletely baked yeast-raised products (included the so-called Brown 'N Serve rolls) have shown that the foods are not heat-damaged. Slight oiling off is noted after the flash heating of the luncheon meats and cheeses. Crystals of sorbic acid are also readily noted on the surface of these foods. However, after over-night storage free oil is no longer apparent and sublimed crystals of sorbic acid no longer visible, having been absorbed into the foods. The flash-heat treatment is so mild that surface discoloration of raw meats does not occur. The appearance of some products covered with crystals of sorbic acid may be objectionable, particularly when the crystals are not readily absorbed into the food, *viz.*, prunes, strawberries, and chocolate cake, when so processed. Other products may also fail to absorb the sorbic acid crystals but remain acceptable in appearance because the color of the foods permits the sublimed sorbic acid to escape visual detection. The product, Brown 'N Serve rolls, is an excellent example of such an application. During the baking in the home the sublimed sorbic acid on the surface of the rolls is volatilized, but this is of no concern since from this time on there is no further need of fungistatic protection. For practically complete volatilization of sorbic acid from the Brown 'N

Serve rolls, the cellophane overwrap should be removed from the package prior to baking.

A feature of this flash sublimation of sorbic acid from wrapper onto food is that the appearance of the wrapper can serve as a good indicator of sufficient heating in volatilizing the fungistatic acid. Thus, when using cellophane or cellulose acetate wrappers treated with sorbic acid and a minimal amount of binder material, these wrappers change in appearance from a translucent white to a crystal clear film as a result of volatilizing the layer of fungistatic acid crystals. The wrappers with the thermoplastic wax coating in high concentrations also exhibit transparency when hot, but become cloudy on cooling due to the resetting of the non-volatile wax base. Changes in transparency are not visually detectable when the fungistatic glassine wrappers are employed.

In general the flash heating of sorbic acid–treated wrappers has been promoted to protect foods which have irregular external surfaces or configurations such that the wrappers containing the fungistatic acid cannot be conveniently placed in intimate surface contact with the wrapped foods. In the flash-heat treatment of the above-mentioned foods less volatilization of sorbic acid occurs from the areas of the sheets in direct contact with the food surfaces. The areas over the air pockets become clear, while those in direct contact with the food surface remain cloudy. This can be ascertained only on examining the wrapper removed from the food. The failure to sublime sorbic acid at the areas of contact of wrapper with food is of no concern since these areas are less vulnerable to mold spoilage and opportunities exist for migration of sorbic acid from these areas on the wrapper into the foods.

SUMMARY

An important consideration in the control of an additive to a food is the purity of the commercially-available material. Sorbic acid as produced on a commercial scale is 100.0% pure $\pm$ 0.5% (one standard deviation), calculated to a moisture-free basis.

In determining expeditious ways for adding sorbic acid to food formulations, advantage should be taken of its greater solubility in certain solvents, such as in ethyl alcohol, in acetic acid, and in aqueous solutions buffered to pH values above 3.1. Values for solubility of sorbic acid in certain systems indicate the maximal quantities which may be added in soluble form to certain foods.

Whereas propionic acid in phase distribution studies is predominantly water soluble and benzoic acid is predominantly fat soluble, sorbic acid falls in between these two as shown by the values for distribution ratio. The ratio of the solubility of sorbic acid in fat to that in water at room temperature (74°F.) is 3.0. The presence of solutes such as sugar and salt, but not acids, is responsible for an increase in this ratio. The lower and more favorable distribution ratio for sorbic acid is responsible in part for the more than three-fold greater effectiveness of sorbic acid in comparison to benzoic acid in protecting foods against mold spoilage, but this cannot explain the four-fold greater effectiveness of sorbic acid in comparison to the propionates.

Advantage can be taken of the ease with which sorbic acid sublimes at elevated temperatures to augment fungistatic protection of foods. A flash heating method has been described, one that has been specially designed to protect foods which have irregular external surfaces or configurations such that the wrappers containing sorbic acid cannot be conveniently placed in intimate surface contact with the wrapped foods.

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SORBIC ACID AS A FUNGISTATIC AGENT FOR FOODS. X. SPECTROPHOTOMETRIC DETERMINATION OF SORBIC ACID IN FOODS IN GENERAL[a]

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(Manuscript received June 30, 1955)

In a previous publication from this laboratory *(2)*, a spectrophotometric method was presented for the estimation of sorbic acid in cheese and cheese wrappers. The sorbic acid in the test sample is distilled at atmospheric pressure along with the water in the test system. This distillation is rendered more efficient by the presence of added magnesium sulphate. The sorbic acid in the distillate is then identified by its characteristic absorption curve and concentration calculated from the reading at the absorption maximum.

This same spectrophotometric method has now been applied to the determination of sorbic acid in many different classes of foods each of which contain products which might require at some time or other supplementation with sorbic acid for protection against mold spoilage.

EXPERIMENTAL PART

In Table 1 are presented data obtained in analyzing 30 foods for sorbic acid content after supplementation of these foods with the fungistatic acid in variable quantities. In analyzing each distillate spectrophotometrically for sorbic acid content, correction has been made for irrelevant light absorbing materials contributed by the same size aliquot of the control unsupplemented product. Calculation of sorbic acid content has been based on the reading at the absorption maximum, on the dilution factor, and on the $E^{1\%}_{1cm}$ value of 2,200 for pure sorbic acid at its absorption maximum *(2)*.

From the values listed in Table 1 it is apparent that recoveries of added sorbic acid are good, averaging practically 100% in this series. Irrelevant absorption in relation to that due to the sorbic acid may vary from as little as 2% to almost as much as 50%. As the sorbic acid supplement in a given food is increased, the relative magnitude of irrelevant absorption is reduced.

In Figures 1 and 2 are plotted the ultraviolet absorption data obtained in analyzing a majority of the food products.[b] The solid line in each graph is the spectrophotometric curve of the test solution containing the distilled sorbic acid, after correction has been made for irrelevant absorbing materials contributed by the same size aliquot of the control unsupplemented food. The broken line is the spectrophotometric absorption curve for pure sorbic acid in distilled water. All curves are plotted in terms of extinction

[a] This paper was presented at the Fifteenth Annual Meeting of the Institute of Food Technologists, Columbus, Ohio, June 13, 1955.

[b] Ultraviolet absorption data on the remaining foods were in agreement with those plotted in Figures 1 and 2.

TABLE 1

Applicability of sorbic acid method for determining the fungistatic agent in foods

Food product	Sorbic acid			Irrelevant absorption in relation to that due to sorbic acid
	Added to food	Found in food	Recovery	
	%			%
Breads, Etc.				
White dough	0.0280	0.0252	90.0	22.5
White bread—whole	0.1000	0.0990	99.0	15.7
White bread—crumb	0.0250	0.0267	106.8	18.5
White bread—crust	0.0250	0.0237	94.8	30.1
Rye bread—crumb	0.0250	0.0269	107.6	12.5
Rye bread—crust	0.0250	0.0255	102.0	17.5
Pumpernickel—crumb	0.0250	0.0272	108.0	10.9
Pumpernickel—crust	0.0250	0.0259	103.6	20.4
Cakes				
Pound cake	0.1000	0.0926	92.6	35.8
Yellow cake	0.2200	0.1961	89.1	24.3
White cake	0.2200	0.2277	103.5	5.8
Chocolate cake	0.2200	0.1918	87.2	34.1
Bread Spreads				
Margarine—100% CM[1]	0.0500	0.0544	108.8	13.8
Margarine— 50% CM	0.0500	0.0465	93.0	12.8
Margarine— 0% CM	0.0500	0.0509	101.8	12.2
Butter	0.0250	0.0246	98.4	27.9
Fruit Juices				
Orange juice	0.1000	0.0969	96.9	10.7
Pineapple juice	0.1000	0.0998	99.8	8.9
Apple juice	0.1000	0.1085	108.5	17.6
Apple cider	0.0250	0.0240	96.0	45.5
Pigmented High Sugar Products				
Chocolate syrup	0.1000	0.0907	90.7	25.5
Maple syrup	0.1000	0.1164	116.4	18.0
Raspberry jam	0.1000	0.1089	108.9	46.8
Strawberry jam	0.1000	0.0978	97.8	38.7
High Protein Foods				
Whole egg	0.1000	0.1020	102.0	9.8
American cheese	0.0880	0.0870	98.9	2.0
Cottage cheese	0.1000	0.0933	93.3	5.1
Chopped beef	0.1000	0.1035	103.5	13.9
Miscellaneous				
Dill pickles	0.0500	0.0504	100.8	5.6
Bacon	0.0350	0.0315	90.0	14.6

[1] CM = % cultured milk in the total milk phase, the remainder being sweet skim milk.

ratios, i.e., the ratio of absorbancy at a given wavelength to that at the absorption maximum. By this method of plotting, ready comparison can be made between the absorption curve of any sample, regardless of dilution of the test material, and that of a product of known purity, for example, pure sorbic acid in water. Light absorption due to extraneous material in the test distillate is plotted in the lower part of each graph in relation to the absorption exhibited by the sorbic acid in the test sample of the same size.

It will be noted in this series of tests that the shapes of the corrected spectrophotometric absorption curves are exactly the same as that of pure

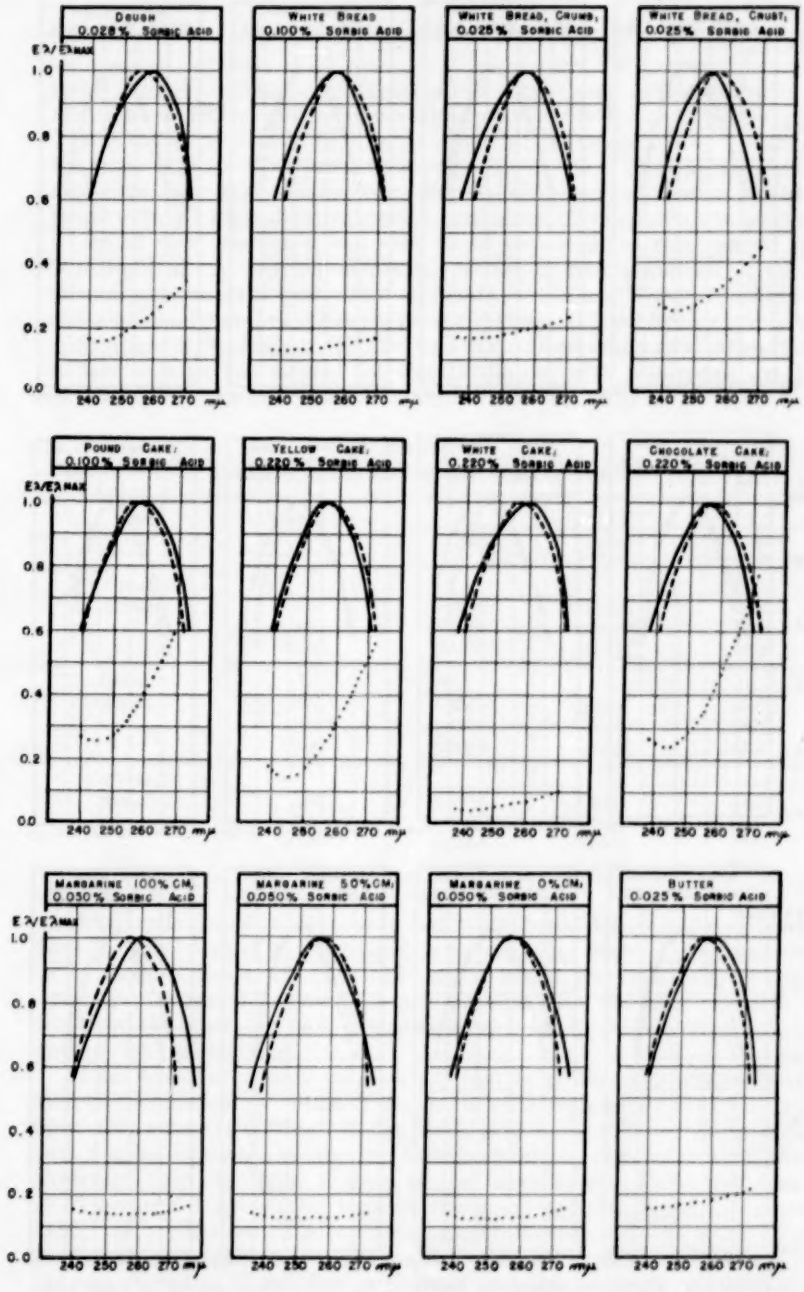

Figure 1. Ultraviolet absorption curves of distillates from samples of bakery products and bread spreads; _____, distillate from product supplemented with sorbic acid and corrected for irrelevant absorbancy; · · · ·, distillate from unsupplemented product (irrelevant absorbancy); – – – –, pure sorbic acid in distilled water (pH 5.9).

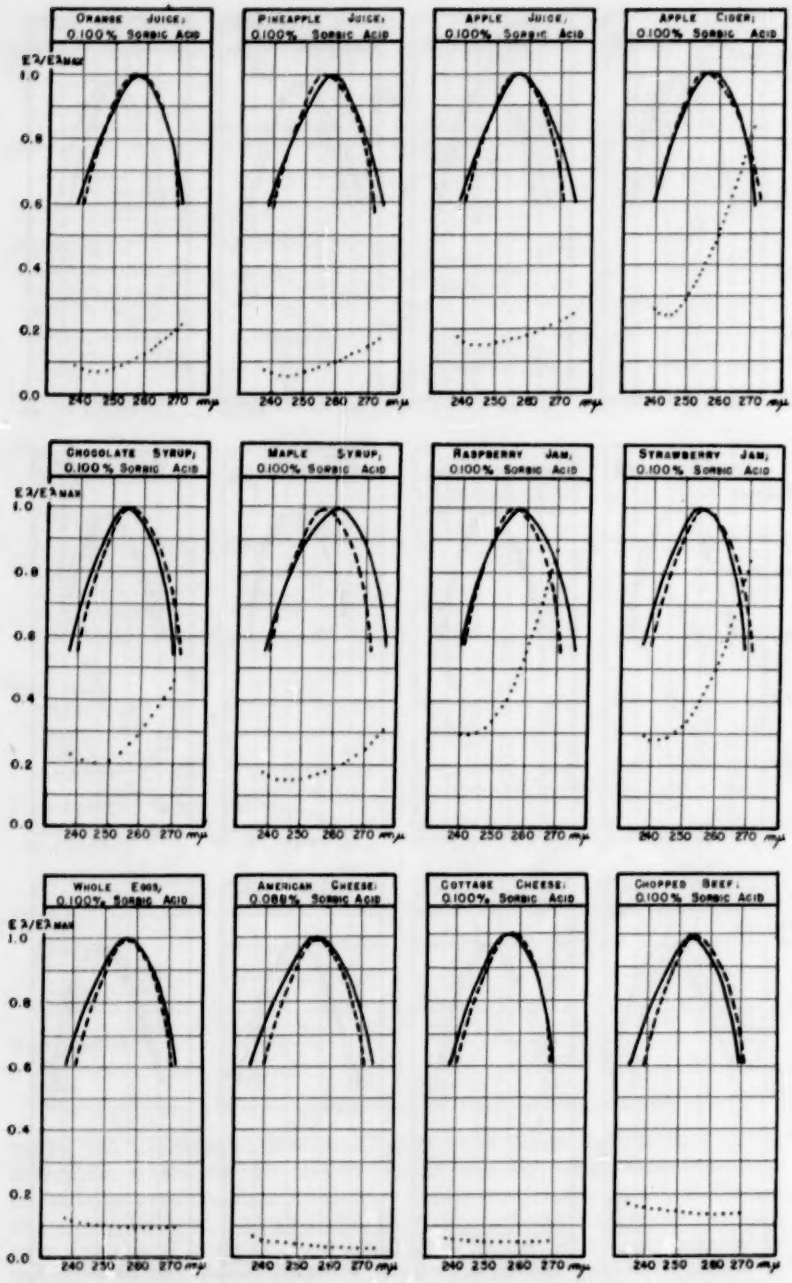

Figure 2. Ultraviolet absorption curves of distillates from samples of fruit juices, syrups, jams, and high protein foods; _____, distillate from product supplemented with sorbic acid and corrected for irrelevant absorbancy; · · · ·, distillate from unsupplemented product (irrelevant absorbancy); – – – –, pure sorbic acid in distilled water (pH 5.9).

sorbic acid. However, the positions of the curves vary somewhat, being to the right or left of the reference absorption curve for pure sorbic acid. This is known to be due to differences in the pH values of the distillates, the more acid the distillate, the greater the tendency for the absorption maximum to be at the higher wave length. Irrelevant absorption has been found to vary both qualitatively and quantitatively from product to product.

In all these studies, it has been found important to keep sample size at a minimum. A large representative portion of the test material is taken, the sample blended[c] with water to obtain a uniform slurry or solution, and a small aliquot then taken for the distillation. It is preferable to depend upon minimal dilutions of the distillate for desired sensitivity rather than to take a large test sample for the distillation with subsequent greater dilution. This is supported by illustrative data in Table 2.

TABLE 2

Effect of sample size on the recovery of sorbic acid from foods

Food product	Product in distilland	Sorbic acid		
		Added	Found	Recovery
	gm.		%	
American cheese	3.00	0.0500	0.0462	92.4
	5.00	0.0500	0.0460	92.0
	10.00	0.0500	0.0311	62.2
Cream cheese	3.00	0.0500	0.0480	96.0
	10.00	0.0500	0.0434	86.8
	15.00	0.0500	0.0332	66.4
White bread	1.00	0.0500	0.0471	94.2
	5.00	0.0500	0.0440	88.0
	10.00	0.0500	0.0413	82.6
	15.00	0.0500	0.0380	76.0

Another factor of importance in sorbic acid methodology is the necessity for pH adjustment of the distilland in testing some foods. If the material to be distilled has a pH above 6.0 and is highly buffered, sulfuric acid must first be added to lower the pH to about 4.5. The data in Table 3 illustrate the need for such a modification in testing whole egg supplemented with sorbic acid.

It has been our recommendation *(2)* that in all research studies blank samples, permitting correction of the spectrophotometric data for irrelevant light absorption, be included in the sorbic acid assay method. In working with a given food on a continuing basis it should be possible to show that irrelevant light absorption is more or less reproducible. From that point on the need for conducting tests on the blank samples would no longer exist; the application of a correction factor should suffice in controlling sorbic acid supplementation of the food. Another factor contributing to the ease of control of this food additive is that the purity of commercially

[c] A Waring blender has proved to be very satisfactory for this purpose.

TABLE 3

Effect of pH adjustment on the determination of sorbic acid in whole egg

Sorbic acid added	pH of distilland	Adjustment of pH of distilland	pH of distillate	Sorbic acid	
				Found	Recovery
%				%	%
0.0250	7.1	none	7.0	0.0191	76.4
0.1000	6.7	none	6.8	0.0654	65.4
0.0250	7.1	to 5.0	6.2	0.0222	88.8
0.1000	6.7	to 4.5	6.2	0.1020	102.0

available sorbic acid is 100.0% ± 0.5% (one standard deviation), calculated to a moisture-free basis *(1)*.

SUMMARY

The spectrophotometric method for determining sorbic acid in cheese has been extended to the analysis of foods in general. These included breads, cakes, bread spreads, fruit juices, syrups, jams, meats and miscellaneous foods, supplemented with 0.025 to 0.220% sorbic acid. Correction is made for irrelevant light absorbing materials contributed by the food by conducting the same test on the same size aliquot of the control unsupplemented food. The importance of keeping sample size at a minimum and depend upon minimal dilutions for desired sensitivity is supported by experimental findings. Of comparable importance is the need of adjusting the distilland to an acid reaction, if the pH of the material to be distilled has a pH above 6.0 and is highly buffered.

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SPORULATION IN *CLOSTRIDIUM PASTEURIANUM*[a]

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(Manuscript received July 11, 1955)

Degenerative changes in laboratory cultures of butyric acid forming bacteria have been described by a number of workers, among them Winogradsky *(8)*, Grimbert *(3)* and, more recently, Kutzenok and Aschner *(5)*. In each case the organism lost the ability to form either spores or clostridial forms. After several transfers in laboratory media, cultures weakened and stopped growing. Unterkofler, Christensen, and Fulmer *(7)* failed to obtain spores from liquid cultures of butyric clostridia grown in culture tubes. To induce sporulation they recommend drying cultures on sterile soil, or exposing to air in thin layers.

Knaysi *(4)* and Brunstetter and Magoon *(2)* concluded that sporulation in *Bacillus mycoides* is inhibited by the acid products of fermentation. Leifson *(6)* considered that a neutral to slightly acid reaction gave best conditions for spore formation by *Clostridium tetani*, *Clostridium botulinum*, *Bacillus vulgatus*, and *Bacillus circulans*. In the case of *Cl. botulinum* the acid reaction resulting from the fermentation of dextrose appeared to inhibit sporulation.

During investigations of butyric spoilage of canned pears and tomatoes *(1)* difficulty was experienced in growing laboratory cultures of *Clostridium pasteurianum*, and in obtaining crops of spores for heat resistance tests. The problem has been studied, and a convenient means of culturing *Cl. pasteurianum* developed. Good yields of spores are obtained by this method.

METHODS AND RESULTS

The culture of *Cl. pasteurianum* used was isolated from canned Flemish Beauty pears showing butyric fermentation *(1)*. Colonies picked from dextrose agar and tryptone glucose agar plates produced acid, gas, and heavy sediment in 3% dextrose broth containing 0.8% yeast extract. However, subsequent transfers in the same medium resulted in a gradual weakening of the culture until at the third to fifth transfer no growth occurred. Microscopic examination showed no spores or clostridia even after prolonged incubation. These and all subsequent cultures were held in the Brewer jar to insure anaerobic conditions.

The pH of the dextrose broth medium was lowered from an initial value of 7.0-7.4 to 4.1-3.95 as a result of fermentation. It was thought that this might be a factor in the weakening of the culture and failure to form spores. Accordingly, in order to neutralize the acid metabolites, an excess (1%) of sterile calcium carbonate was added to an actively growing dextrose broth culture, which was then reincubated. Microscopic examination showed only rod forms (Figure 1). The pH of the medium at this stage was 4.5. After 24 hours, a preponderance of well developed clostridia was observed. After 48 hours the majority of cells contained spores (Figure 2). These later appeared free of the sporangia. The pH of the medium during this latter period was 5.2-5.4.

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Figure 1. Clostridium pasteurianum —96-hour dextrose broth culture without calcium carbonate. pH 4.5. Cells show polar staining or slight swelling only.

Repeated transfers of *Cl. pasteurianum* using spore suspensions prepared in the manner outlined were made over a period of 18 months. In all cases spores developed within 48 hours. On the other hand, no spores developed in dextrose broth without calcium carbonate, regardless of the length of incubation.

Effect of cations. To study the possibility that calcium or other ions might stimulate spore formation, a number of calcium and other salts were aded in 1% and 0.1% concentration to actively growing 48-hour dextrose broth cultures of *Cl. pasteurianum* which were then reincubated. The salts used were calcium sulphate, calcium chloride, calcium citrate, calcium lactate and calcium gluconate, magnesium carbonate, magnesium hydroxide and magnesium sulphate, sodium citrate and sodium bicarbonate, and potassium nitrate.

Microscopic examination at 24-hour intervals over a period of 14 days showed no spores in any of the cultures so treated. The final pH developed was from 4.05 to 4.5. Control cultures with 1% calcium carbonate added showed profuse sporulation within 48 hours, the final pH being 5.3-5.5.

Effect of pH. To test the effect of pH on sporulation in the absence of calcium, a number of cultures of *Cl. pasteurianum* were grown in wide mouth screw capped jars (2 × 2 in.) each at a constant pH. The pH values selected were 4.5, 4.7, 5.0, 5.3, 5.5, 5.7, 6.0, and 6.3. The pH of each culture was measured at 24-hour intervals and adjusted with normal sodium hydroxide. The use of wide mouth jars permitted insertion of the electrodes during this operation. Sterilization of the electrodes with quaternary ammonium compound (Roccal) and rinsing with sterile water prevented contamination of the cultures. Microscopic examination was made daily over a period of one week.

In the intervals between additions of sodium hydroxide, sufficient acid was produced

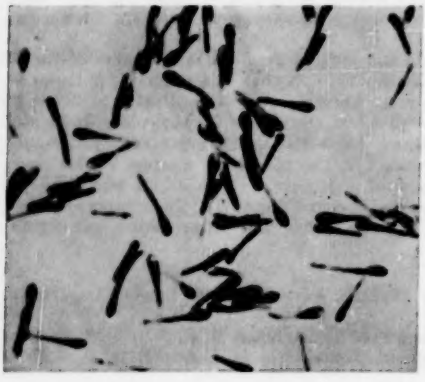

Figure 2. Clostridium pasteurianum —96-hour dextrose broth culture showing sporulating cells. One per cent of calcium carbonate added after 48 hours incubation. pH 5.2-5.4.

to lower the pH of each culture by approximately 0.3 pH units. The minimum pH value recorded was 4.3.

Between pH 4.3 and 4.7 spore formation was largely inhibited, and only a few spores were produced between 4.7 and 5.0. Between pH 5.0 and 5.7 clostridia developed within 24 hours. Spores appeared in fairly large numbers within 72 hours. After 4 to 6 days the culture was composed largely of sporulating cells. Between pH 6.0 and 6.3 a few spores appeared after 60 hours, but the concentration of spores remained low indicating that some inhibition took place in this range.

Effect of cations and controlled pH. To find if spore formation was affected by calcium or magnesium in cultures maintained at constant pH, dextrose broth cultures containing 0.2% of added calcium chloride, calcium sulphate, and magnesium sulphate were neutralized as before with normal sodium hydroxide, at 24-hour intervals. Spores appeared within 72 hours as in the previous series in the pH range 4.9-5.5. At pH 4.6 or lower no spores were observed.

To determine the optimum buffering concentration of calcium carbonate, 24-hour dextrose broth cultures of *Cl. pasteurianum* were prepared, and to these were added 1, 2, 3, 4, and 5% calcium carbonate. The pH values of the cultures before treatment were 4.45-4.6. Immediately after treatment the pH values rose to 5.7-5.9, but were stabilized at 5.45-5.7 within 24 hours.

Microscopic examination before addition of calcium carbonate showed no spores or clostridia. Within 24 hours clostridia predominated with a few spores appearing. After 48 hours spores were seen to be present in nearly all cells. The concentration of spores appeared to be the same in all preparations, containing 1 to 5% calcium carbonate.

DISCUSSION

Results obtained indicate that the accumulation of acid fermentation products is the principal factor involved in the cessation of growth and failure of *Cl. pasteurianum* to form spores in liquid culture. Neutralization of the culture solution with normal sodium hydroxide at 24-hour intervals during incubation so as to hold the pH between 5.0 and 5.7 permits the formation of numerous clostridia and spores within 72 hours. However, the accumulation of acid in the periods between additions of sodium hydroxide appears to delay sporulation.

Calcium carbonate, by its continuous neutralizing and buffering action, does not allow the pH to fall below the optimum range of 5.45 to 5.7. As a result, spores begin to form within 12 hours, reaching a maximum concentration within 24-48 hours.

Calcium or magnesium ions have no effect on spore formation. When salts of these metals were present, untreated broth cultures failed to produce spores, as was the case in the same medium without calcium. On the other hand broth containing calcium or magnesium salts, when neutralized, produced spores at the same rate as did neutralized broth without calcium. Sodium citrate and bicarbonate and potassium nitrate have no effect on spore formation.

Laboratory cultures of *Cl. pasteurianum* could not be grown in sugar broths by continuous transfer of vegetative cells. By the addition of 1% sterile calcium carbonate to an actively growing culture, followed by reincubation for 24-48 hours, spore formation was stimulated. Only by transferring spores so obtained has it been possible to maintain cultures in vigorous condition.

SUMMARY

The failure of *Clostridium pasteurianum* to form spores in liquid culture appears to be a result of the accumulation of acid metabolic products.

Under these conditions laboratory cultures weaken and fail to grow after 3-5 transfers. However if the pH of the medium is held within the range 5.4-5.7, optimum conditions for spore formation are produced. The addition of an excess of sterile calcium carbonate, approximately 1%, provides a buffering effect, maintaining the pH at the optimum point for spore formation. Calcium or magnesium ions as such have no effect on spore formation.

Acknowledgment

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BITTER FLAVOR IN CARROTS. I. A TENTATIVE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF BITTERNESS[a]

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(Manuscript received July 15, 1955)

In recent years a serious flavor problem has arisen which seems to be connected with the storage of carrots prior to processing. These off-flavors are usually characterized as bitter.

There are only a few reports in the literature dealing with off-flavors in carrots. Yamaguchi, Howard, and McNelly *(7)* found a lower alpha carotene content in bitter carrots than in non-bitter samples and suggested that the bitter material may be breakdown products from carotenoids. Hervey, Robinson, and Schroeder *(3)* showed that carrots develop an astringent off-flavor which was most pronounced in samples infected with aster yellow virus. Newcomb and Alderman *(5)* found that on storage, carrots from muck soils were more susceptible to oxidative rancidity than upland grown carrots. Although no bitter compounds have been isolated from carrot roots, Cizycki and Hermanns *(1)* reported the isolation of a sirupy, bitter glycoside from carrot leaves; Reeb *(6)* found a bitter glycoside, named daucusin, in seeds of red carrots. Since neither of these preparations were crystalline there would appear to be some question about their purity. Furthermore, their characterization has been incomplete; the material from the leaves is believed to contain a carbohydrate, a phenolic aglycone, and a volatile aldehyde moiety. Even fewer data are available on the chemical composition of the material from seeds.

To obtain a clearer understanding of the factors involved in the development and control of bitter flavors in carrots we have undertaken an investigation which has as its goal the isolation, identification, and objective determination of compounds responsible for the bitter taste. It was observed that hydrocarbon extracts from bitter carrots had ultraviolet absorption spectra different from those of non-bitter samples. We have investigated the possibility of using this observation as a basis for an objective method of estimating the bitterness of carrots and are reporting here results obtained with more than 200 processed carrot samples.

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[b] Beech-Nut Packing Co., Canajoharie, N. Y.

[c] The authors wish to thank the staff of the Food Chemistry Laboratory of the Beech-Nut Packing Co., Canajoharie, New York, for their varied contribution to this project.

EXPERIMENTAL

Raw material. Forty-eight strains of Chantenay, Danvers, Nantes, Imperator, Supreme Half Long, Berlicum, and Flakkee varieties were grown on a sandy soil at Greece, N. Y. Thirty-two of these strains were also grown on both muck and loam soil near Geneva, N. Y. All carrots were harvested and graded in the first week of November, 1954. Only U. S. No. 1 carrots were used in these experiments. Samples of all lots were placed in a commercial cold storage at 32.5°F., 13 lots were placed in common storage at 40° to 45°F., while 26 lots were processed within 2 weeks after harvest. Stored carrots were canned in the first 2 weeks of January, 1955.

Processing procedure. Six kg. samples were washed and boiled for 5 minutes to loosen the skin. They were scraped by hand and then diced. Following thorough mixing the diced material was placed in No. 2, "carrot-enamel" cans and enough water added to fill the containers. They were then closed under a vacuum with a commercial sealer which automatically regulated the head-space. The cans of carrots were cooked at 240°F. for 40 minutes, and then cooled in cold water.

Organoleptic scoring. The flavor evaluation was carried out by a panel of 15 to 20 tasters, selected for their ability to detect bitterness in carrots. The samples were prepared for tasting by passing them through a kitchen grinder, followed by thorough mixing. The panel judged the flavor of 4 unknown samples, using a scale from 1 (most bitter) to 10, against a non-bitter comparison standard which was arbitrarily assigned a score of 8. The standard was prepared from one large lot of carrots harvested and canned in November. With this procedure the differences in the scores for duplicates did not exceed one unit.

Spectrophotometric procedure.[4] The entire content from one No. 2 can was treated in a blender for 5 minutes. Then, 5 g. were placed in a 60 ml., ground-glass stoppered bottle and 40 ml. solvent were added. Solvent with an end absorption below 230 mμ was prepared from practical n-hexane or Skellysolve-B by passing the liquid through a silica gel column *(2)*. To avoid differences in the degree of extraction it was found desirable to adopt a standardized procedure consisting of shaking each bottle vigorously three times for 15 seconds. Twelve samples were prepared at a time and the spectrophotometric data were obtained within one hour of the time the samples were shaken. Optically clear solutions were obtained on decanting the supernatant. Readings were taken at 290, 265 and 240 mμ with a Beckman Spectrophotometer Model DU, using 1 cm. cells. The spectrophotometric data are expressed as "Height of the 265 mμ peak" and were calculated with the following formula:

$$\text{opt. dens. 265 m}\mu - \frac{(\text{opt. dens. 290 m}\mu + \text{opt. dens. 240 m}\mu)}{2} \times \frac{\text{ml. solvent}}{\text{g. sample}}$$

Duplicate analysis of a large number of samples showed that the results are reproducible within 5%.

RESULTS

When bitter carrots are extracted with petroleum ether a bitter, orange residue is obtained upon evaporation of the solvent. This extract differs from those from non-bitter carrots, not only in its taste characteristics but also in its ultraviolet absorption spectrum. As can be seen from Figure 1, Curve I, the extract from non-bitter carrots has the typical carotenoid absorption in the visible range and little absorption in the ultraviolet region above 220 mμ. The extracts from bitter carrots however, Figure 1, Curves II and III have pronounced peaks in the ultraviolet. In all the extracts from bitter carrots which we have compared the 265 mμ peak had a higher absorption than the 290 mμ peak and both always occurred together.

Taste panel evaluation was compared to ultraviolet absorption in known mixtures of bitter and non-bitter carrots. From Figure 2 it may be seen that changes in the

[4] The authors acknowledge with thanks the technical assistance of Miss Joanne E. Whitcombe in the spectrophotometric determinations.

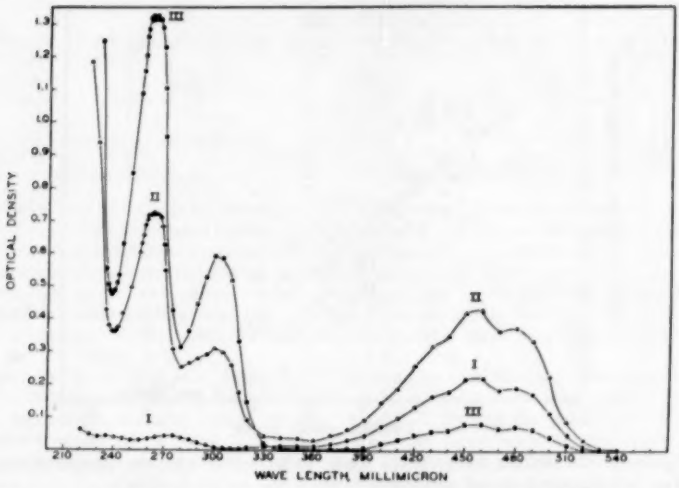

Figure 1. Absorption curves for hexane extracts from processed carrot samples. Legend: I (·—·) = extract from non-bitter carrots. II (▲—▲) = extract from bitter carrots. III (■—■) = extract from very bitter carrots.

proportion of bitter carrots can be detected by both procedures; a high organoleptic score and a low value for the height of the 265 mμ peak denote lack of bitterness. For this particular experiment the taste test procedure was less accurate, since the panel could not distinguish between samples containing 50% and 75% of the bitter carrots.

Further information on the degree of correlation between the flavor scores and the spectrophotometric data was obtained from the analysis of 200 different samples, Figure 3. The taste panel scores varied from 8.5 to 1.3 and the height of the 265 mμ peaks

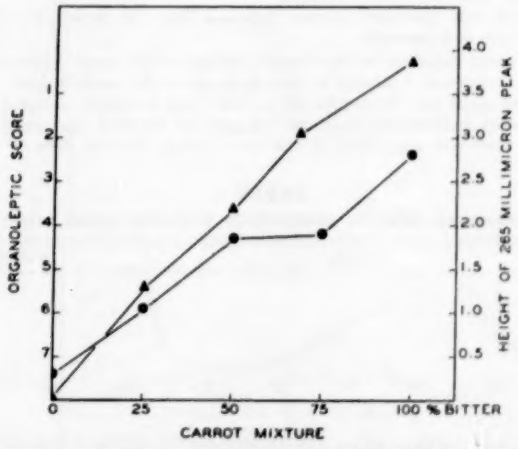

Figure 2. Taste panel scores and spectrophotometric data for known mixtures of non-bitter and bitter carrot samples. Legend: ●—● = taste panel scores; ▲—▲ = spectrophotometric data.

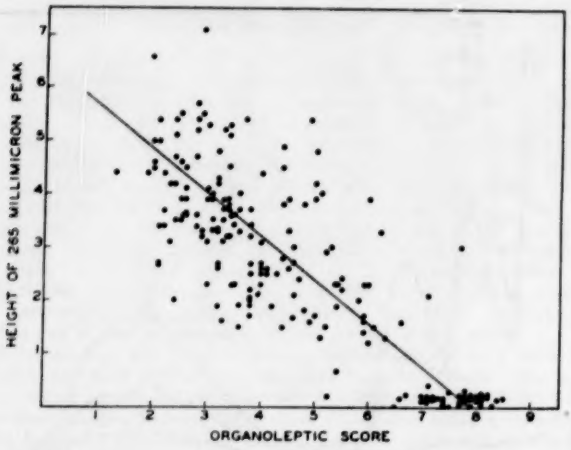

Figure 3. Correlation between the taste panel scores and the spectrophotometric data for 200 processed carrot samples.

from 0.0 to 7.1. The correlation coefficient for the flavor scores and the spectrophotometric data was −0.82. Using the line of best fit, the average of the two regression lines, it could be shown that the spectrophotometric data of 68% of the samples agreed with the taste panel scores within ± 1 unit. Agreement between the spectrophotometric data and the subjective scores was better for the less bitter samples. Thus, for samples with a taste panel score above 5.5, the spectrophotometric analyses of 90% of the samples agreed within ± 1 unit with the results obtained by the taste panel.

As a further confirmation of the relation between bitter flavor and the "height of the 265 mμ peak" we have analyzed 3 pairs of commercially prepared carrot samples which had been selected by their manufacturers as being typically bitter and non-bitter. As the results in Table 1 show all the non-bitter carrots had very much lower values for the 265 mμ peak than the bitter samples.

Limited work with uncooked carrots indicates that the procedure outlined here is also usable for the raw material.

Due to the great variation in the "height of the 265 mμ peak" observed in different lots of carrots it seemed desirable to obtain data on the peak heights for individual carrots from the same lot. Therefore 25 carrots were randomly selected from each of the 3 lots, canned individually and the "height of the 265 mμ peaks" determined. These samples were not submitted to the taste panel. As the data in Table 2 show,

TABLE 1

Spectrophotometric data for commercially prepared, pureed carrot samples

Sample	Height of 265 mμ peak
Company 1	
Non-bitter	0.2
Bitter	3.3
Company 2	
Non-bitter	0.5
Bitter	3.0
Company 3	
Non-bitter	0.5
Bitter	2.8

TABLE 2
Height of 265 millimicron peak for 25 individually packed carrots from 3 lots

Lot	Average peak height	Mean variation	Maximum peak height	Minimum peak height
1	0.24	± .06	0.4	0.1
2	4.8	± 2.6	18.6	1.3
3	4.1	± 1.8	6.6	2.1

large variations in the size of the peaks for the individual carrots were observed in 2 out of the 3 lots. This heterogeneity has of course a direct bearing on the sampling problem.

We have also obtained evidence that the substance(s) responsible for the ultraviolet absorption is not evenly distributed throughout the carrot. When the phloem, the outer portion, and xylem were separated and canned, it was found that the phloem had a spectrophotometric reading of 2.7 whereas that for the xylem was only 0.7. The taste panel scores also showed more pronounced bitter flavor for the phloem portion, 3.2, than for the xylem, 5.4.

Examination of Figure 3 shows that a few samples were found in which agreement between flavor scores and spectrophotometric data is particularly poor. One such pair which had almost identical values for the "height of the 265 mμ peak," 3.2 and 3.1, had flavor scores of 6.2 and 2.3, respectively. The breakdown of the relationship between bitter taste and "height of the 265 mμ peak" prompted us to investigate this case more fully. Figure 4 shows the absorption curves for the two hexane extracts. It can be seen that the maxima and minima occur at the same wave lengths. This is consistent with the view that the ultraviolet absorption peaks in both cases are due to the presence of the same substance(s). One difference between the ultraviolet absorption of the extracts from the bitter and non-bitter carrots shown in Figure 3 is the higher intensity of the absorption for the bitter extract. Whether or not the substance(s) responsible for this absorption is involved in the bitter flavor development is not known.

DISCUSSION

From the data reported here it seems probable that there is some relationship between the flavor scores and the ultraviolet absorption of

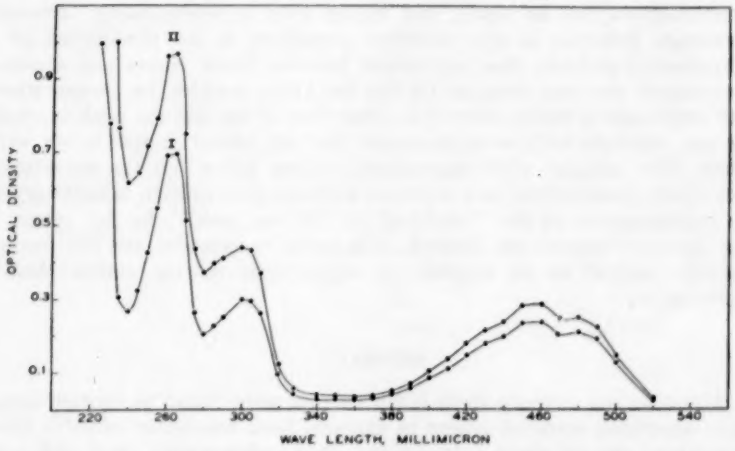

Figure 4. Absorption curves for extracts of two carrot samples with similar values for the "height of the 265 millimicron peak" and different taste panel scores. Legend: I = absorption curve for the sample with a flavor score of 6.2. II = absorption curve for the sample with a flavor score of 2.3.

the hydrocarbon extracts of processed carrots. However, the exact nature of this relation is not known at present. From the data available it would appear that the compound is not the main contributor to the bitter off-flavor. This belief is based on the observation that extracts from a few carrot samples with greatly different organoleptic scores have nevertheless similar ultraviolet absorption and that a few samples were found among those shown in Figure 3 where the height of the 265 mμ peak was markedly different, but the taste panel scores very similar. It is concluded therefore that the substance(s) with the absorption peak at 265 mμ is an index compound which in many cases gives a useful indication of the degree of bitterness of processed carrots. Until the ultraviolet absorbing material has been isolated and its taste characteristics determined, it is of course impossible to decide whether or not the compound is bitter. Work is in progress on its isolation and identification.

From Figure 3 it can be seen that there are only 4 samples that did not have an absorption peak at 265 mμ but that there were 38 extracts which had relatively small absorption maxima. It is quite likely that this weak absorption in the ultraviolet region is contributed by carotenoids, many of which have peaks in this region *(4)*. That this absorption is small is seen from Figure 1, Curve I where the height of the ultraviolet peak is less than 7% of the total absorption at 455 mμ. It is therefore possible that a minor contribution to the 265 mμ peak absorption is made by the carotenoids and that samples with peak absorption of 0.3 or less may actually be free of the compound(s) usually associated with bitter off-flavors.

A number of points must be considered in the application of the spectrophotometric determination to the objective estimation of bitter flavor in carrots. The spectrophotometric procedure has distinct advantages over taste panel scoring by virtue of its simplicity, the rapidity with which determinations can be made, and by its good reproducibility. Another advantage, inherent in any objective procedure, is the elimination of a comparison standard. Best agreement between flavor scores and spectrophotometric data was obtained for the less bitter samples, i.e., preparations with organoleptic scores above 5.5. Therefore if the 265 mμ peak is small, one can conclude with some assurance that the carrot sample is not very bitter. For samples with organoleptic scores below 5.5 the correlation with spectrophotometric data is poorer and therefore caution is necessary in the interpretation of the "height of the 265 mμ peak." In this range, if very accurate results are desired, it is safest to consider the spectrophotometric method as an adjunct to organoleptic scoring rather than a replacement.

SUMMARY

Hydrocarbon extracts from bitter carrots were found to contain ultraviolet-absorbing material absent in extracts from non-bitter carrots. Good correlation was obtained between the spectrophotometric data and taste panel scores of 200 samples. The application of the photometric measurements as a method for the objective estimation of bitter flavor in carrots is discussed.

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FOUNDATION OF FOOD PROCESS CALCULATION METHODS[a]

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As one would expect, constant refinement and improvement of procedures have marked the mathematical methods of evaluating canned food processes during their years of use. Until very recent years, proposed improvements have met with generally harmonious reception. Within the last 7 or 8 years, however, some differences in viewpoint have been expressed as to what should be regarded as a sound principle in respect to distribution of lethal effect within a container of food and to the significance of this distribution as a criterion of sterilization of the food. We believe that a discussion of logic in connection with the fundamental elements related to this subject is desirable. To present such a discussion is the purpose of this paper.

PROBABILITY OF SURVIVAL OF MICROORGANISMS

In a book by the authors, soon to be published, is an exposition of a mathematical procedure for evaluating processes with the number of surviving microorganisms per container serving as the criterion of sufficiency of heat treatment.

Two separate aspects of that type of method, which are, to a considerable extent, independent of one another, have recently been subjected to considerable discussion by workers in the field. The first is concerned with the logic of using the number of surviving microorganisms per container as the criterion of sufficiency of heat treatment. The second involves the validity of a basic assumption that has been accepted in the authors' method referred to above. We shall first discuss this assumption.

There would appear to be no basis for disputing the fact that the distribution of surviving spores indicates the quantities of lethal heat to which the organisms in different portions of the food in a container are

[a] This discussion of the foundation of process calculation methods constitutes Chapter 3 of a completed manuscript of a book on the general subject of evaluating the effects of lethal energy upon organic cells. This chapter is being published in advance of the publication of the book in response to a request to the authors that this be done. The authors trust that certain references to other chapters of the book will not prove distracting to the reader. Some such references have been deleted but it has seemed necessary to retain others, notably Chapter 15, in order to preserve the integrity of the discussion. At the same time, space limitations do not permit an expansion of the retained references in order to provide for the reader all of the material that is closely related in substance to this chapter, which would be available to a reader of the book. It is believed that the discussion as presented, however, is sufficiently replete with explanation to make it fully understandable.

subjected. Acceptance of this fact is essential to any method of calculating sterilizing processes.

Stumbo *(15, 16)* suggested that a special interpretation of his concept on distribution of surviving spores be incorporated as a vital element in such calculation procedures. By this special interpretation, the point or region of greatest probability of survival is shown not to be the point that receives the smallest amount of sterilizing heat action, which point, herein, is designated as the *critical point*. This fact is said to justify the abandonment of the use of the heat effect at the critical point as the criterion of sufficiency of a process and to establish the sole validity of the number of surviving microorganisms per container as the criterion of sufficiency. Stumbo later *(17)* outlined a procedure using this criterion without placing special significance on the point or region of greatest probability of survival but using the same method of calculating the distribution of surviving spores as was presented in the earlier papers *(15, 16)*. A different basis of calculation is used in a calculation procedure recently developed by the authors.

The nature of the difference will be pointed out later, along with an explanation of a reason for preferring the authors' method when the calculation applies to sterilization.

To calculate the survivors, Stumbo imagines the contents of the container to consist of a collection of portions that constitute a series of nested shells, designated as iso-*F*-value shells, which are defined by the condition that all material which constitutes any one shell receives equal lethal heat treatment. As the series progresses from the outside surfaces of the container to the shell that contains the critical point, there is a progressive decrease in lethal value for the successive shells. Stumbo applies the survivor[b] curve to each shell independently, basing the calculation of survival on the entire volume of the food substance which constitutes the shell as the unit volume; in other words, considering each shell as defining its own unit volume. No two shells have the same volume; therefore, unit volume for the calculation for each shell is unique to that shell.

We shall consider the logic of the significance ascribed by Stumbo to the calculated distribution as a factor in the criterion of sterilizing sufficiency.

Stumbo defined the region of greatest probability of survival as the region occupied by the shell in which the number of survivors would be greatest according to the calculation. His calculation showed that the shell occupying the region of the greatest probability of survival, according to the above definition, is not the shell within which is the point of lowest degree of lethal treatment. He suggested that the latter point may not be critical in consideration of the efficacy of a sterilizing process and that the effect observed at that point may not be a true criterion of sufficiency of the sterilization of the contents of the container.

One's viewpoint in regard to Stumbo's concept depends upon the assumptions that one is prepared to accept. His concept on the probability

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[b] The name "survivor" for this curve was adopted by agreement among workers in the field of bacteriology and food technology. It represents a slight change from the term "survival curve," which appears in D. E. Lea's *Actions of Radiations on Living Cells*. Macmillan Co., New York, 1947.

of survival appears to be sound only when associated with the following assumptions:

a. Differences in intrinsic individual heat resistance of microorganisms exist and constitute a critical factor.

b. The probability of high resistance within any portion of substrate is related, by a proportionality factor, to the volume of substrate being considered.

We believe that the following discussion will clarify the necessity for including these assumptions in Stumbo's hypothesis.

Each iso-F-value shell is treated as a unit, to which are applied an initial heat resistance value which depends upon the above probability concept and F value which is consistent with the heat penetration properties of the material. Calculations, then, as shown by Stumbo, reveal that the region of greatest probability of survival may be, in fact, generally is, away from the location of least lethal effect.

It must be emphasized that acceptance of this concept carries with it the requirement that the distribution of initial resistance be strictly in accordance with the above assumptions. The use of the location of the least sterilizing value, to provide a criterion for sufficiency of a process, is based upon the assumption that the probability that the microorganism of highest heat resistance will be present at the location of least sterilizing value is at least great enough to justify a process that is capable of destroying the organism of maximum heat resistance when that organism is in that location. The latter assumption would seem to be a more logical one for conduction heating products than for convection heating products; however, the assumption seems fairly well justified when considering any product in which solid portions remain stationary, regardless of convection currents. All calculation methods developed by the authors are based on the assumption that the initial heat resistance of the vulnerable entity is the same in every unit volume of the contents and that all unit volumes are of uniform size. Modifications to take into account, on a practicable basis, the Stumbo idea on distribution of initial resistance present formidable obstacles.

CRITERION OF SUFFICIENCY OF PROCESS

Mathematical methods which have been generally used for evaluation of sterilizing processes have employed the lethal effect at the point of least lethal effect as the criterion of effectiveness. This criterion has served well during the years of its use—since 1920—when calculations were based upon what was thought to be the point of least lethal effect (the center of the container). The question of whether or not the number of survivors per container provides a more logical basis for judging effectiveness of sterilizing processes than the number of survivors in the unit of volume which includes the point of lowest amount of lethal heat is inseparably linked with the question of whether or not the region of greatest probability of survival is that which includes the point which is subjected to the least lethal energy.

In undertaking a critical examination of the logic of the case, we shall discuss the following interrelated questions:

1. What hypotheses are used to substantiate the concept that the number of residual viable spores per container provides a sound basis for process evaluation?

2. Does spoilage following heat processes occur in direct proportion to the number of residual spores per container or per unit volume that can be shown to be viable immediately after the process or do other factors determine the amount of spoilage?

3. Does concentration of spores have an effect on their individual resistance to heat?

4. If the answer to 3 is positive, at what minimum concentration, if any, does this effect cease to exist?

5. If, at the end of a process, culture tests by a standard procedure show a concentration of N viable spores per unit volume or per container, would inoculation of sterile food with N fresh spores per unit volume or per container give the same growth results in the food as are given by the N viable spores which have been heated?

6. As an analogue to 5, if a process, by calculations based upon a known rate of destruction, leaves M residual viable spores per container or per unit volume but these spores produce no growth under a standard method of culturing and no spoilage in the food, would inoculation with M fresh spores per container or per unit volume give no growth in the food?

Let us follow usual custom and designate F value of a process for the unit volume that contains the point of least lethal effect by the symbol F_c and the composite F value for the entire container as F_s. F_c is based on the maximum residual concentration per constant unit volume within the container; F_s is based on the residual concentration per container, regardless of the size of the container.

Having defined F_c and F_s, we may now add to the list what may be the crucial question:

7. Will the number of cans to spoil be the same for No. 1 as for No. 10 cans when the value of F_c for the two is the same or when the value of F_s for the two is the same? Alternately, will the relationship between the number of No. 1 cans to spoil and the number of No. 10 cans to spoil depend on either the relative values of F_c or the relative values of F_s?

Until the above 7 questions, and others, are answered, we shall not be justified in concluding that either the F_c or the F_s basis is the correct one for use in comparing sterilizing effects in different containers or of different processes.

An answer to question No. 1 should be found in the literature. Going to Stumbo *(15, 16, 17)*, Hicks *(2, 3)*, and Gillespy *(1)*, we seem to find that the justification for using the number of residual viable spores per container as the criterion of the value of a process comes from the belief that the location in which the spores have the greatest probability of survival is not the location that receives the smallest quantity of lethal heat. Entertainment of the latter concept, therefore, is usually accompanied by a belief in the validity of using F_s as the sole basis for process evaluation.

Let us now see whether or not an excursion into logic will reveal further answers.

PROXIMITY OF RESIDUAL VIABLE SCORES

If initial proximity of viable spores to each other is a deciding factor affecting individual heat resistance, the F_c basis is the more likely to be the correct one; if proximity is not an important factor, the F_s basis may be, but is not necessarily, the correct one. If proximity is not important, the principle, which we accept, of logarithmic rate of destruction of spores (this point will receive further attention later), means that there must be a fixed distribution of individual resistances to heat among the spores which originally contaminate a food, regardless of the concentration of the spores.[c] By virtue of this fixed distribution of resistances, the maximum resistance of individual spores in a collection which constitutes a contaminant must increase indefinitely as the concentration of the spores increases and thus the number of spores on every level of resistance must increase at an absolutely fixed rate, in relation to concentration, as the concentration of spores is increased. This rate of increase in the various levels of resistance with increase in numbers is such that the rate of destruction is logarithmic; that is, the proportions of spores at all levels of resistance are continuously maintained at such values that, as the spores on levels of successively increasing resistance are progressively killed, the rate of destruction is logarithmic.

Until it can be proved as to which of these factors, proximity of spores to each other or fixed ratios of individual resistances, is responsible for the logarithmic rate of destruction of contaminant spores, or as to the extent to which either factor contributes to this result, we can only be guided by conjecture and base our calculation procedures on the factor that seems best to fit the facts. To the authors, the proximity factor seems more likely to control the rate of destruction than does the individual

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[c] In the sense in which the term "individual resistance" is here used, the term implies resistance which is essentially inherent and, consequently, essentially permanent or prescriptive to the individual spore. Adventitious differences between individuals, which may affect the resistance of individuals temporarily, are in a different category of properties. The latter are of the type which may depend upon proximity of spores to each other or upon other similar fortuitous conditions, such as would be expected to control rates of reaction in case permanent differences in individual resistance do not exist.

The adventitious influences are analogous to the change in states of molecules, to which is attributed the control of rates of chemical reactions, such as the loss or acquisition of electrons, as postulated by Proctor *et al. (12)* to explain the effects of cathode rays on organic cells, and such as change in magnitude of energy or in geometrical orientation of molecules. All of these casual influences are here considered to be in the same class as the factors for which physical association of spores with each other would be responsible.

A factor related to "individual resistance" is age, which, it seems, must play some part in determining when an organism will succumb to heat. Suppose we assume that every micro-organism of a given strain will succumb at the same age if subjected throughout the period of its viable existence to a given, rigidly controlled, pattern of influencing conditions; that is, if every organism is subjected to exactly the same pattern of temperature-time relationship and to exactly the same substrate conditions. Acceptance of this assumption may be entirely consistent with an exponential rate of destruction of spores in an inoculum. The influence of age should be directly related to the influence of concentration, for it seems reasonable that the spread in ages of the organisms in a collection will increase with increase in numbers of organisms.

resistance factor—except as age of organisms influences the latter factor —just as the concentration of molecules in a solution controls the time required for the completion of a chemical reaction involving those molecules. If proximity is the deciding factor and if the effects of this factor upon the rate of destruction continue to prevail regardless of how low the concentration of spores becomes, it would seem logical that the power of growth of the spores might also be dependent upon their proximity to each other. If so, we believe that logic would dictate that the end point of a process be decided on the basis of concentration of residual viable spores per unit volume because there would have to be a minimum point in concentration per unit volume at which growth of microorganisms in a food becomes impossible. When this minimum concentration is reached, there will be no spoilage, regardless of how many containers have residual theoretically viable spores or how many such spores there are per container.

Holloman and Fisher *(4)*, on a nucleation concept, advanced the theory that a colony of cells, in a given volume of substrate, must be of a critical size, or larger, in order for the cells to survive and grow. These authors suggested that either one of two hypotheses, seemingly equally tenable although converse to one another, could account for this phenomenon. Their presentation supports the theory that the capability of cells to grow is influenced by their proximity to one another in the medium.

If the germination of residual viable spores were influenced both by proximity of spores to each other and by the number of residual viable spores per container, there would be a higher rate of germination in a large can than in a small one even though the F_c value were the same for both cans because there would be more residual viable spores in the larger container than in the small one.

Although the *end point*, designated by either the F_s or the F_c value, is defined as the point at which a given theoretical low concentration of residual viable spores, such as .007 spores per container or .00005 spores per unit volume, is reached, it is assumed, in connection therewith, that none of the residual viable spores will germinate and cause spoilage. It is assumed, in other words, that destruction has proceeded to a point at which spores that are capable of germinating exist only theoretically and not in reality.

If proximity has nothing to do with the power of the spores to germinate, and, if the food is a good culture medium for the bacteria, we must assume that the only reason residual viable spores will not germinate is because they have individually lost the power to do so, due to some effect of the heat. If this is the explanation, we must conclude that, to reach the point of no spoilage, each spore on the highest resistance level must be subjected to a definite heat effect which is sufficient to render it incapable of germination. If these spores of maximum resistance level are as constant in their heat resistance characteristics as is assumed on the basis of the "distribution of individual resistance" hypothesis, each of these spores, in whatever container, must have received a definite and equivalent heat treatment as a minimum. This makes the end point depend upon the heat treatment at the point in the container of minimum heat treatment, which brings us to the same basis of decision as does the

proximity hypothesis; namely, that the end point of a process should be decided on the basis of concentration of residual viable spores per unit volume of material (units of uniform volume, of course).

Experimental studies have been reported *(1, 2, 3, 15, 16, 17)*, and such studies are continuing, for the purpose of establishing whether or not spoilage results substantiate the F_c hypothesis or the F_s hypothesis. One must presume that only extensive statistical analysis will lead to a justifiable conclusion; therefore, it is to be expected that a period of years will elapse before a conclusion, based upon these tests, can be justified.

Regardless of the outcome of these studies, two new calculation methods recently developed by the authors will still be valid. One of these methods is based on the use of critical point survivor data as the criterion of process sufficiency (the F_c hypothesis), while the other is based on the use of survivor data of the container as a unit (the F_s hypothesis). The latter method amounts simply to an extension of the former, since the determination of the number of surviving spores in a container consists of an integration procedure applied to the survivor data for all portions of the food in the container. Thus, the result for the container is obtained through a summation of results found by the former method for points distributed throughout the container.

We trust that we have succeeded in showing the relationship existing among the older and the newer concepts involved in the mathematical treatment of canned foods processes. Later, we shall again look at the question of whether the F_c or the F_s value constitutes the logical criterion of sufficiency of a process. In that discussion, some specific assumed conditions to illustrate the lines of reasoning will be used.

CONCEPT OF THERMAL DEATH TIME

The term, thermal death time, was created to designate the time required to destroy, by heating at a given temperature, all microorganisms capable of spoiling the food. "Destroyed" microorganisms are those which are rendered incapable of growing, as indicated by chosen methods of testing growth. The newer concept of thermal death time is that, in a theoretical sense, the organisms are not all killed since the survivor curve which shows the relationship between heating time and the number of survivors is a semi-logarithmic curve that never reaches zero on the survivors scale, regardless of how long the heating is continued. The introduction of this concept into our calculation procedures brought with it the necessity of establishing an end point by arbitrarily choosing a point on the survivor curve which represents destruction of microorganisms to a degree which obviates any growth of the organisms in the food. Thus, under the new concept, we may still use the term "thermal death time" in referring to the time required to heat the microorganisms at a chosen temperature in order to achieve a desired result, and the term may still be defined as the time required to destroy all microorganisms capable of spoiling the food. The change that has taken place is that we now hold in reserve the idea that some microorganisms are still theoretically viable although, to the best of our knowledge, they are incapable of spoiling the food. The change in concept has come simultaneously with a revolution in laboratory tech-

nic for determining destruction data. The procedure of counting survivors to determine the rates of destruction and to establish survivor curves seems to be replacing the older thermal death time technic of counting "positive" and "negative" containers and establishing thermal death time as the minimum time at which "positive" containers are no longer obtained.

It should be stressed that this change in concept of an end point of a process has no effect whatsoever upon calculation technics for the evaluation of processes. Regardless of what end point is chosen, the considerations from the standpoint of treatment are the same. The name of the thermal death time curve may be changed to "thermal destruction time" curve if we wish, but it still is the same type of curve, showing the relationship between heating time and heating temperature necessary to attain the condition which is called sterility or to attain a condition which represents a specified degree of approach toward sterility. It does not matter whether the selected end point is one viable organism per ml., one viable organism per container, one per million containers, or one per sextillion containers, it is still an end point and is handled mathematically by any procedure which deals with the old "thermal death time" as an end point. Nor does this infer that the procedure must be one which applies to a critical point only. It can be an end point procedure applying to an entire container full of food.

NATURE OF SURVIVOR CURVE

The calculated residuum of viable spores is based on the survivor curve —the curve that shows the relationship between the numbers of surviving organisms, expressed as per cent of the original number, and the dose of the lethal agent. Curve B in Figure 1 is one form of a survivor curve.

For ionizing radiations, the dose is usually expressed in rep (Roentgen-equivalent-physical); for heat, the dose would logically be expressed in terms of degree-minutes or degree-seconds. But degree-minutes or degree-seconds, applying to different temperatures, are not linearly additive; therefore, it is more convenient to use time alone as the unit of the dose scale and to let each curve represent a single temperature of heating. Dose for ionizing radiations could be similarly divided into factors of intensity and time for use in plotting separate survivor curves representing different levels of intensity in rep per hour with time alone serving as the unit of the dose scale.

In his chapter on "Target Theory," Lea *(5)* deduced an exponential equation for the survivor curve, which he designates "survival curve," based on single unit action by rays, that is, ionization produced by a single hit on each target. Under this hypothesis, the first hit is invariably effective and sterilization is accomplished when the last surviving target (spore) receives its first hit. Lea believes that only the single unit action hypothesis can account for an exponential survivor curve if bacterial cells or spores of the same type and strain have uniform resistance in the given substrate. He believes the exponential relationship in destruction might result from cumulative action of rays only if the resistance of the individual organisms varied in accordance with a certain skew pattern of distribution.

It is not our purpose to go into a speculation on the merits of Lea's

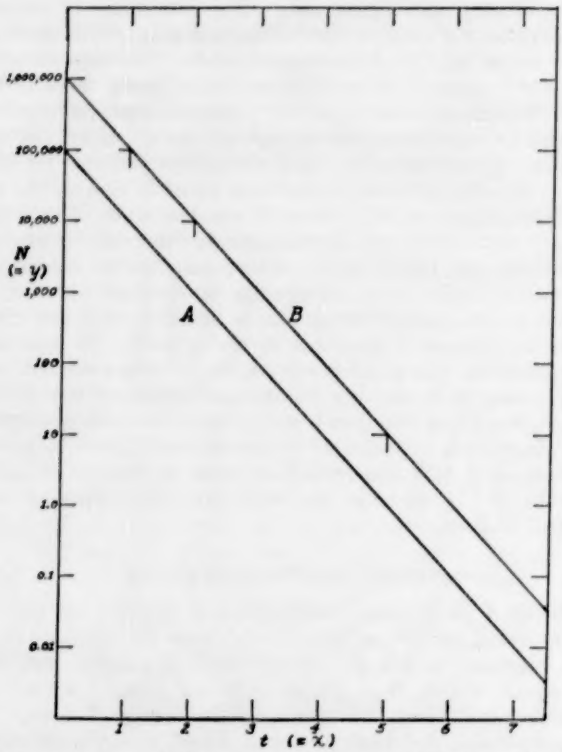

Figure 1. Survivor curves to illustrate meaning of probability of survival. N = number of viable bacterial cells per unit volume. t = time of heating in minutes.

viewpoint, especially since he does not apply it to heat as the lethal agent. We point out, however, that Lea appears to believe that the true survivor curve in ionizing radiation is exponential; at the same time he attempts to describe the conditions under which the sigmoid characteristic might be expected to appear. Since the questions discussed by Lea are the same as those which repeatedly arise over the shape of the survivor curve resulting from heat treatment of bacterial spores, one seems justified in assuming that similar chemical and physical laws are operative in cell destruction by heat as in cell destruction by ionizing radiation. Just how closely the mechanical devices of destruction resemble each other in the two cases we shall not attempt to say. However, there now appears to be a considerable degree of concurrence among bacteriologists on the fact that injury is inflicted on bacterial spores by lethal heat which is not sufficient to inactivate them—injury which leaves the spores weakened so that they are either retarded in germination or more prone to succumb to any unfavorable conditions. This fact seems to constitute evidence in favor of the cumu-

lative theory of destruction by heat and against the single unit action theory. If the latter theory applied to heat destruction, it would seem that the spore would be either completely destroyed when it receives a "hit" or would remain fully vital when it receives no "hit." We shall not attempt, however, to describe what constitutes a "hit" in heat sterilization.

Acknowledging the fact that insufficient evidence is available to establish the precise nature of the survivor curve in heat destruction of spores, we feel that it is not unreasonable to believe that perfect experimentation will eventually show this curve to be truly exponential—without the sigmoid characteristic which has appeared at times in experimental results. We may be influenced, of course, by our wish to entertain this belief because it simplifies the accommodation of the survivor curve to our process calculation procedures. Nevertheless, we believe the true survivor curve to be a straight line when plotted to semi-logarithmic coordinates except that there may be a lag phase at the beginning of the destruction treatment producing a deviation from the straight line at that location. All strictly mathematical procedures for evaluating sterilizing processes are based on this assumption.

Morowitz *(8)* presents an argument in favor of the exponential form of survivor curve from ionizing radiations and develops an equation for the curve. Data of Morgan and Bohrer *(7)* and Pratt and Ecklund *(10)* on ionizing radiation of spores show exponential relationships except for lag periods at the beginning of the destruction period. Proctor and Goldblith *(11)*, Nickerson, Proctor, and Goldblith *(9)*, and Stern and Proctor *(14)* show, for ionizing radiations, survivor curves having the exponential relationship without lag periods. Stumbo, Murphy, and Cochran *(18)* present experimental evidence for the exponential survivor curve for spores subjected to heat. Schmidt *(13)* points out that the available evidence favors the exponential relationship.

DEFINITION OF POINT OF GREATEST PROBABILITY OF SURVIVAL

The question as to the proper criterion of sufficiency to use in calculating sterilizing processes is one of major importance to the fulfillment by this book of its function. Therefore, an analysis of the definition of the location of greatest probability of survival seems essential. For the purpose of logical presentment, the following conditions are assumed: In the contents of a container, the iso-F-value portion that contains the critical point, has a volume of 1 and contains 10 viable spores. In an iso-F-value shell, having volume of 1,000, there are 10,000 viable spores. The container is subjected to a heat process in which the spores in the portion of food having a volume of 1 suffer lethal heat effect expressed as $F = 2.1$, which, according to the survivor curve, destroys 97.14% of the spores. The spores in the portion of the food having a volume of 1,000 suffer lethal heat effect expressed as $F = 3.4$, which, according to the survivor curve, destroys 99.726% of the spores. In the first portion of the food (that containing the critical point), 0.286 spore survives, or one spore in each $3\frac{1}{2}$ containers. In the second portion of the food, 27.4 spores survive (0.274% of 10,000) in each container.

Obviously, according to the survivor curve, more spores survive in the second portion of the food under heat effect $F = 3.4$ than in the first portion of the food under heat effect $F = 2.1$. Does this fact constitute evidence of a greater probability of survival in the second portion than in the first portion? We shall consider this question first on the basis of the assumption that every spore has the same resistance to heat. In this case, each of the 27+ spores that survive in food portion No. 2 entered the process with the same resistance as the spore that survives in every third or fourth container in food portion No. 1. Still, the 27+ spores survived a destroying power 62% greater than that which destroyed all spores in food portion No. 1 in at least two containers out of every three. What imparted to the 27+ spores the power to survive this process? Their concentration and environment originally were identical to those of the spores in food portion No. 1 which were almost all destroyed by a less severe heat treatment. These facts would seem to indicate that, in practice, all spores individually do not have the same resistance to heat.

Let us assume then, for further illustration, that some spores of a given pure suspension have greater heat resistance than others in the same suspension. Suppose that, among the 10,000 viable spores in food portion No. 2, there are 100 spores having resistance at the maximum level and that this level is the same as that of the resistance of all spores in the previous illustration. Since the volume of food portion No. 2 is 1,000 times as great as that of food portion No. 1, by the laws of probability, we have a right to believe that there is one spore having resistance at the maximum level in food portion No. 1 in every tenth container, on the average. Now, we find that 27+ spores out of the 100 highly resistant spores (27+%) in food portion No. 2 escape destruction in a process of $F = 3.0$, whereas, in the food portions No. 1 of each ten containers, the spore of maximum resistance plus about two spores of lower than maximum resistance escape destruction in a process of $F = 1.9$. This seems to make sense.

Let us proceed to a third illustration and assume that, among the 10,000 viable spores in food portion No. 2, there are 500 spores having resistance at the maximum level. Probability indicates that there is one spore having resistance at the maximum level in food portion No. 1 in every second container, on the average.

Under these circumstances, we find that 27+ spores out of the 500 highly resistant spores (5.4%) in food portion No. 2 escape destruction in a process of $F = 3.2$ and, in food portion No. 1, 57.1% of the spores (4 spores in each 14 containers, which initially contained 7 spores) having resistance at the maximum level survive after a process of $F = 2.0$.

As a final illustration, let us consider the condition in which there are, in food portion No. 2, 1,000 spores having resistance at the maximum level and, according to probability indications, one spore having resistance at the maximum level in food portion No. 1 in each container, on the average. Here, 27+ spores out of the 1,000 highly resistant spores (2.7%) in food portion No. 2 escape destruction in a process of $F = 3.3$ and, in food portion No. 1, 28.6% of the spores (one out of every 3½) having resistance at the maximum level survive after a process of $F = 2.05$.

Now we arrive at the necessity of deciding which food portion, No. 1 or No. 2, marks the location of greatest probability of survival. Is it the portion in which the greater number of spores survive (portion No. 2) or the portion in which the higher percentage of the spores of maximum resistance survive (portion No. 1)? In the above three illustrations involving variable individual resistance of spores, the percentages of survival of the spores at the maximum level of resistance in portion No. 2 were 27.4, 5.4, and 2.7, respectively, while, in portion No. 1, the percentages of survival of the spores at the maximum level of resistance were 100, 57.2, and 28.6, respectively.

The answer to our question would appear to rest in one's interpretation of the definition of probability. Owing to the importance of this decision to accepted concepts of methods of calculating processes for sterilization, two illustrative considerations of the last of the above illustrations, bearing on its interpretations, will be given. It is recalled that, in that illustration, 1,000 spores among the 10,000 spores in food portion No. 2 have heat resistance at the maximum level. To facilitate the reasoning, we shall assume that the spores having resistance at the maximum level in food portions No. 1 are uniformly distributed so that there is one such spore in each container. Here we are confronted by the proposition that existed in the illustration involving the assumption that every spore has the same resistance to heat. Every spore having resistance at the maximum level has the same resistance. Each of the 27+ spores that survive in food portion No. 2 in each container entered the process with the same heat resistance as the spore that survives in every third or fourth container in food portion No. 1. Still, the 27+ spores survived a destroying power approximately 60% greater than that which destroyed all spores in food portion No. 1 in at least 2 containers out of every 3. What imparted to the 27+ spores the power to survive this process? Their concentration and environment originally were identical to those of the spores in food portion No. 1, 71.4% of which were destroyed by a less severe heat treatment. Our concept of the relationship existing among (a) the heat resistance of microorganisms, (b) the amount of lethal heat applied, and (c) the amount of destruction accomplished breaks down completely under these circumstances.

For a new set of conditions in the final illustration given above, we conceive a comparison between the above food portion No. 2, having a volume of 1,000 units and a third food portion having the same volume (1,000 units) which embodies, in its interior, the previously described iso-*F*-value shell called food portion No. 1, having a volume of 1 unit and containing the critical point in the contents of the container. Food portion No. 3 consists of a sufficient number of iso-*F*-value shells to make up the volume of 1,000 units and none of these iso-*F*-value shells is that which constitutes food portion No. 2. In other words, there is no overlapping of the food portions being considered; therefore, the iso-*F*-value shell which constitutes food portion No. 2 completely encloses food portion No. 3 without touching it.

In the heat process, the various iso-*F*-value shells of food portion No. 3 have various *F* values, going upward from the 2.05 value of food portion No. 1. The *F* value of the outermost iso-*F*-value shell of food portion No. 3

is less than the 3.3 value of food portion No. 2. Thus, the mean F value of food portion No. 3 lies between 2.05 and 3.3. Food portion No. 3 enters the process carrying the identical flora of microorganisms to that of food portion No. 2. Therefore, under our well established concept of the relationship existing among (a) concentration of spores, (b) resistance of spores, (c) amount of lethal heat applied, and (d) amount of destruction accomplished, there will be more survivors in food portion No. 3 after the process than in food portion No. 2. By any interpretation of the definition of probability, one must conclude that there is a greater probability of survival in food portion No. 3 than in food portion No. 2. This conclusion is at odds with the interpretation which would place the location of the point of greatest probability of survival in food portion No. 2.

There appears to be an impasse. However, the incongruities that have appeared seem to dissolve if one adheres to a simple principle embodied in the definition of the survivor curve.

The solving of this problem is a fantastically complex exercise in logic because of the number of assumptions that must be taken into account. The above illustrations may reveal how easily one can reason himself around a circle.

To elucidate the principle just referred to in the definition of the survivor curve, we shall resort to two examples. First, let us recall to mind three facts about the unit volume of substance. These are (a) all unit volumes are of the same size, (b) a unit volume may have any size but is preferably very small since heat is assumed to penetrate it with infinite rapidity, and (c) there may be any number, including a fractional number less than unity, of unit volumes in a "portion" of food chosen for use in these examples.

Example 1. It should be self evident that, in a quantity of food that has been thoroughly homogenized and mixed, the probability that the number of spores in a unit volume will equal the average number for all unit volumes is the same for all unit volumes. Likewise, if the heat resistance of individual spores varies, the chance that a given unit volume will contain a spore having resistance at the maximum level is the same for all unit volumes. Therefore, if one puts a quantity of the food having a volume of 1 into a container and a quantity having a volume of 1,000 into another container and subjects every unit volume of each portion of the food to a heat process having an F value of 6, there will be a greater probability that a spore will survive in a portion of food having a volume of 1,000 than in the portion having a volume of 1. In fact, even though every unit volume of the former portion should be subjected to a heat process having an F value of 7 while the latter portion was subjected to a process of $F = 6$, there might still be a greater probability that a spore will survive in the former portion than that one will survive in the latter portion. This is because of the fact that the chance that here will be a spore having resistance at the maximum level in food portion of volume 1,000 is 1,000 times as great as the chance that there will be such a spore in food portion of volume 1.

Example 2. If example 1 is duplicated in a world in which all spores are identical individually in respect to heat resistance, when every unit volume of food material in the two portions is subjected to a heat process of $F = 6$, the probability of survival in the second portion will be as great as that in the first portion. This is revealed to us by the survivor curve, which applies separately to each unit volume of food material. However, let us take two portions of the food material, each having a volume of 1, place one portion in a container, then thoroughly mix in another container the other portion with 999 unit volumes of food material which is identical to the previously used material except that it is sterile. Here, there is the same number of spores in each portion but one portion has a volume of 1 while the other portion now has a volume of

1,000. If every unit volume of both portions of food is subjected to a heat process of $F = 6$, a spore in the first portion having a volume of 1, will have a vastly greater probability of surviving than will a spore in the second portion. This is revealed to us by the survivor curve which tells us that, if we have 2 unit volumes of food material, one containing 1,000 times as many spores as the other and the spores are identical individually in heat resistance, it will take longer to destroy the spores in the first unit volume than it will take in the second.

In the rationalization of this problem, reasoning sometimes follows a course such as to indicate that the true significance of numbers of cells, as applied to survivor curves, is not realized. In the equation,

$$x = D\ (\log y - \log y_o) \qquad (1)$$

which represents the survivor curve, y and y_o are sometimes described as designating numbers of viable cells before and after heating. Actually, by definition of the survivor curve, y and y_o designate concentrations of viable cells. To merely use the word "numbers" in describing y and y_o does not depict the true nature of the survivor curve. To illustrate, let us consider the two portions of food described in example 2. Each portion has the same number of viable spores but the concentration of spores in the first portion is 1,000 times as great as the concentration in the second portion. Therefore, if y for the first portion is 1 million, y for the second portion is 1,000. These values would have to be substituted for y in the equations of the respective survivor curves. If a third portion of the original food material were taken, having a volume of 1,000 units, the value of y in the survivor curve applying to this portion would be the same as that for the survivor curve of the first portion, viz., 1 million, although the total number of viable spores in the portion, containing 1,000 volume units, would be 1,000 times 1 million, or 1 billion. Thus, survivor curves with the same value of y would apply to the first portion containing 1 million viable spores and to the third portion containing 1 billion viable spores, while, applying to the second portion, containing 1 million viable spores, would be a survivor curve in which the value of y is the quotient of the y value of the first curve, divided by 1,000.

These examples are intended to show that if a survivor curve is applied to two different portions of food material when a quantitative relationship in respect to survival of microorganic cells is to be established between the two food portions, the principle of expressing the quantity y in terms of concentration and not simply in terms of numbers must be observed. Otherwise, the relative significance of numbers of survivors in different portions of a food mass is obscured. If number of survivors in a portion of food is considered in an isolated sense, without respect to its quantitative relationship to survivors in another portion of food, it is permissible to express y in terms of numbers without respect to concentration.

It is agreed by all that the criterion of a calculation must depend upon the probability of survival of a single spore having the capability of germinating in some portion of the food. If there is an iso-F-value shell having a volume of 1,000 units located in a container a distance from the central axis equal to one-half the radius of the container, there is a large number of food portions, each having a volume of 1,000 units, located nearer to the center of the container than the given iso-F-value shell. Any one of these food portions, after a process, contains a greater number of survivors or, is more likely to contain one survivor, than the given iso-F-value shell and the portion that contains the greatest number of survivors, or is most likely to contain one survivor, is that which contains the point in the food mass which suffers the least effect from the lethal agent.

Of all principles involved in the problem under consideration, failure to observe this principle of survivor curves is most often responsible for fallacious reasoning. We repeat: the true criterion rests in the question

at what location in the food mass is the point at which a spore is most likely to survive, retaining the capability of reactivation? Again, we answer: *that point is the one which is subjected to the least lethal effect.*

Survivor curves represent the results of experiments on the rate of destruction of viable microorganisms. A curve thus established may be used in comparative relationships only when applied to the same microorganism and the same substrate as those used in the experiment and with a concentration of viable organisms per ml. (y value) which is represented by some point on the curve. The point on the curve representing this concentration designates zero time on the scale, in other words, it designates the beginning of the heating period. Therefore, a survivor curve representing the results of a test on a microorganism having an initial concentration of N spores per ml. is applicable to this microorganism when the initial concentration is N spores per 1,000 ml. by letting the point on the curve representing a concentration of $N/1{,}000$ spores per ml. designate zero time, or the beginning of the heating period. To illustrate, the survivor curve B shown in Figure 1 may be applied to the microorganism for which it was established when the initial concentration of viable spores is 1 million per ml. by designating as zero time the point on the time scale marked 0 minutes, when the initial concentration of viable spores is 10,000 per ml. by designating as zero time the point on the time scale marked 2 minutes, or when the initial concentration of viable spores is 10 per ml. by designating as zero time the point on the time scale marked 5 minutes. The possibility of lag in rate of destruction at the beginning of the heating period is here neglected by assuming that the curve is straight, beginning at zero time.

According to the definition of the survivor curve, therefore, when such a curve is applied to the iso-F-value shells, or iso-j-value regions, within the contents of a container, the same value of y must be used in the survivor curve equation as it is applied to all shells or regions. The authors adhere to this principle in the calculation procedure developed by them[d] and they believe that departure from this principle cannot be justified.

To return briefly to the matter of defining the location of the point of greatest probability of survival, we trust that we have made it clear that this definition must depend upon one's interpretation of the definition of probability in this application. To the authors, the point of greatest probability of survival is the point which is subjected to the least effect of lethal heat, which is referred to as the critical point. There would seem to be no rational justification for accepting any other interpretation except under one condition. This is the premise that the chance that a spore having resistance at the highest level being present at the critical point is so remote that the hazard of neglecting it is inconsequential. We are not prepared to accept this premise.

RELATIVE VOLUMES OF ISO-F-VALUE SHELLS

The postulation of a progressive increase in initial heat resistance of spores in the iso-F-value shells, going from center to periphery of a con-

[d] To be presented in Chapter 15 of their book. See footnote[a].

tainer, has already been mentioned. This hypothesis depends upon the assumption that the iso-*F*-value shells considered in order from center to periphery, increase in volume progressively. The hypothesis is essential to, but is not a precursor of, an acceptance of the possibility that the point of greatest probability of survival is not the point that suffers the least effect from the lethal agent.

It can readily be proven that, in the Stumbo concept, the location of the so-called "point of greatest probability of survival" depends directly upon (a) the rate of heat penetration and (b) the relative magnitudes of the volumes chosen, the designation of which, for the present purpose, may be changed to iso-*mean* *F*-value shells because the *F* value may not be uniform throughout the volume of the shell. Thus, the nominal *F* value of the shell would be the mean of the *F* values throughout the shell. Without presenting such proof, the authors are usurping the right to show, in Figure 2, what they think the relationship is among the three factors, rate of heat

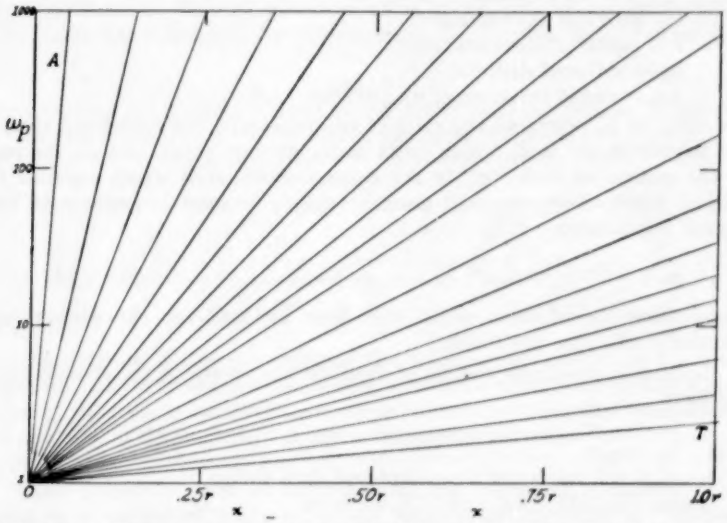

Figure 2. Influence of rate of heat penetration and of relative magnitudes of the volumes of the iso-F-value shells upon the location of the shell which contains the greatest number of survivors. Each curve represents a fixed rate of heat penetration. ω_p = ratio of peripheral iso-F-value shell to that of the iso-F-value shell at the critical point. x = radial distance from critical point axis to the iso-F-value shell having the greatest number of survivors. A indicates low rate of heat penetration. T indicates high rate of heat penetration. r = radius of the container. Each curve represents a rate of heat penetration intermediate to rate A and rate T.

penetration, shell volume, and location of the shell having the greatest number of survivors.

The construction of Figure 2 is based entirely upon reason. Mathematical proof of the relationship is not attempted because we are satisfied that logic alone is sufficiently convincing of the fact that the location of the iso-*F*-value shell that will contain the greatest number of survivors

can be changed at will by varying the volume relationship among the shells. Stumbo *(15)* showed that the location varies with the rate of heat penetration.

The volume relationship can be varied, of course, by varying the thickness of the shells. For instance, if it is desired that the peripheral shell have less volume than the volume element that contains the critical point, this condition can be brought about by making the side wall and end thicknesses, Δx and $\Delta \bar{x}$, respectively, of the outermost shell sufficiently small to make the desired relationship between volumes exist.

Assuming that each iso-*F*-value shell is a perfect cylinder with plane ends, the volume of a shell (walls only—not the volume enclosed by the shell) can be shown, by solid geometry, to be as indicated in eq. (2).

$$v_a = \frac{3\pi l}{r} x_a^2 \, \Delta x_a \qquad (2)$$

where v_a = volume of walls of shell "*a*."
l = length of the container.
r = radius of the container.
x_a = radius of shell "*a*."
Δx_a = radial thickness of wall of shell "*a*."

We shall let ω_p represent the ratio of the volume of the peripheral shell to the volume of the shell, which contains the critical point, and ω_a, the ratio of the volume of shell "*a*" to the volume of the shell which contains the critical point. Now, we shall assume (simply because it appears to be a logical relationship) that

$$\omega_a = 1 + \frac{x_a}{r} (\omega_p - 1) \qquad (3)$$

[Any other logical form might have been assumed for (3) without substantially affecting the result.]

Letting v_o equal the volume of the shell that contains the critical point, we have

$$v_a = \omega_a v_o \qquad (4)$$

also $$v_p = \omega_p v_o \qquad (5)$$

where v_p = the volume of the peripheral shell walls.

From (2), (3), (4), and (5), we have

$$\Delta x_a = \frac{v_a}{3\pi l x_a} \left(\frac{r}{x_a} + \omega_p - 1 \right) \qquad (6)$$

Eq. 6 is helpful in enabling one to comprehend the variation in magnitude of Δx with variation in volume of the iso-*F*-value shells. For instance, for a No. 10 can (603 × 700), when the volumes of all shells are identical ($\omega_p = 1$), Δx of the shell having a radius of 0.01 inch is almost 96,000 times as great as Δx of the peripheral shell. If the radial wall thickness, Δx, of the shell having a radius (to the midpoint of the shell wall) of 0.01 inch is 0.02 inch, the thickness of the peripheral shell would be approximately 21×10^{-8} inch. According to (6), if $v_a = 1$, the radial thickness of the peripheral shell is

$$\Delta x_p = \frac{\omega_p}{3\pi l r} \qquad (7)$$

in other words, Δx_p is directly proportional to ω_p and is inversely proportional to $3\pi lr$, which is approximately the surface area of the peripheral shell. This is exactly the surface area of the peripheral shell when $l = 2r$, i.e., when $\Delta x = \Delta z$. When $\omega_p = 1$ (all shells are of equal volume), Δx for all iso-F-value shells is inversely proportional to $\frac{3\pi lx^2}{r}$, which, when $l = 2r$, is the surface area of the shell. As ω_p increases in magnitude, Δx for all iso-F-value shells approaches the state of being inversely proportional to $3\pi lx$, which, when $l = 2r$, is r/x times the surface area of the shell. This relationship is controlled by the assumed relationship between ω_a and ω_p [see (3)].

By (6), a ratio of 1:1 between Δx_p (radial thickness of the peripheral shell) and Δx_a is obtained only when $\omega_p = \infty$. As long as ω_p has a finite value, the ratio value will be practically that of $3\pi lx_a$.

Ratios calculated from (6) between Δx_p and Δx_a for $x_a = 0.01$ inch are given in Table 1. Eq. (6) gives the value of Δx_p after the substitution of symbols having subscript $_p$ for the corresponding symbols having subscript $_a$.

TABLE 1

Ratios of radial wall thickness of the peripheral shell to radial wall thickness of shell "a" when the radius of shell "a" equals 0.01 inch in the No. 10 can ($\underline{l} = 7$; $\underline{r} = 3.1$).

ω_p	Ratio $\Delta x_p/\Delta x_a$	ω_p	Ratio $\Delta x_p/\Delta x_a$
0.1	954,400	100	1,264
1.0	95,700	1,000	405
2.0	48,000	1,000,000	309
5.0	19,300	∞	1
10	9,860		

The purpose of this discussion of the volume relationship among iso-F-value shells is to create for the reader a realistic conception of the magnitudes of differences involved in these relationships when the relationships are accepted as determining factors in establishing the location of the point of greatest probability of survival.

Table 1 merely gives an indication of the degree of flexibility which exists in a method of determining the location of the point of "greatest probability of survival," in which this location depends upon the relative volumes of a series of nested shells. Either the relative volumes or the relative thicknesses of the shells must be chosen arbitrarily. Table 1 shows how the relative volumes are related to the relative thicknesses. Stumbo chose unity as the ratio of the thicknesses of the shells ($\Delta x_p/\Delta x_a$). This means that the shells are of equal thickness. Another ratio would have been equally logical. For instance, if equal volumes for the iso-F-value shells in a No. 10 can were desired, the ratio of Δx_p to Δx_a would be 95,700.

PRESENT STATUS OF THE CRITERION QUESTION

In his 1953 presentation, Stumbo *(17)* states, "Accurate evaluation can be accomplished only if all points in the container are considered, the result of the heat process as it concerns the whole container being the

sum of effects of all points throughout the container." This thought is invariably associated with the belief that (a) the location of greatest probability of survival should be treated as a critical location and that (b) this location moves about, depending upon the conditions of the process. Therefore, it is to be assumed that these two ideas are postulated in Stumbo's calculation procedure.

The above quoted statement is interpreted to mean that the F_s basis of evaluation should be used in calculations to predict sterilization. (F_s is the F value of a process based upon the survivors of the entire container as a criterion.) The procedure developed in his presentation substantiates this interpretation. He outlined a calculation procedure based on this principle, which principle, without question, is a sound one in its proper place. That the results obtained by a method of calculation based on the F_s hypothesis differ from those obtained by a method based on the F_c hypothesis is shown in comparisons presented both by Stumbo *(17)* and by the authors of the book of which this presentation is to be a part.*

The authors believe that, while the F_s value basis is proper for evaluating the destruction of organoleptic quality, vitamins, and possibly enzymes, where knowledge of the number of survivors per container is the prime objective of the calculation, the critical point basis should be used for sterilization calculations until reliable information shall have become available relative to variations in heat resistance of individual spores. If such information should validate the probability that the spore of greatest heat resistance will not be present in the critical point often enough to cause concern, the question of changing the basis of calculation should then be considered.

In any method of calculation based upon survival within the entire contents of a container, it is immaterial whether or not the principle set forth previously, pertaining to the use of the equation of the survivor curve, is followed. The result will be the same if y is used to represent concentration as it will if y is used merely to represent numbers of cells in iso-F-value shells. On the destruction of all entities except the hazardous ones (bacteria, virus, etc.), percentage survival in the container as a unit probably is the proper basis for calculations.

Stumbo's concept of "location of greatest probability of survival" has been discussed by Jakobsen *(5)* and others.

LOCATION OF CRITICAL POINT

The point which suffers the least lethal heat effect in a product that heats by conduction, is usually in or near the geometrical center of the container. It has been reported by Tischer and coworkers *(19)*, however, that, in some conduction heating meat products, a region shaped like a doughnut around the geometrical center has been found to have the least lethal heat effect. A puzzling pattern of distribution of temperature in the chunks of beef was shown. Distance from the can wall seems to have had a somewhat incidental effect. Lethal values at different times were erratic, insofar as location was concerned. Variation in structure of the product in various

* Chapter 15 contains the comparison referred to.

regions might have been a deciding factor, although one consistent result was that the center point usually had a higher lethal effect than points immediately surrounding the center. While this condition does not affect the procedure for calculating lethal value of a process at a point, it does indicate the importance of knowing the exact location of the critical point for which an evaluation is to be made.[f] That is the question involved in the assumption of Stumbo which was discussed in the preceding paragraphs.

CONCLUSION

Answers to the seven questions posed in the early portion of this chapter, based upon specific experimental evidence, cannot yet be given. Logical deductive answers are possible, however.

Since it is a generally accepted fact that spores are injured by heat in such a way as to affect their capabilities to perform metabolic functions, we may assume negative answers to questions 5 and 6.

Question 1. Literature indicates that justification for using the number of residual viable spores per container as the criterion of the value of a process rests on the hypothesis that the location in which the spores have the greatest probability of survival is not the location that is subjected to the smallest quantity of lethal heat. Logic shows this hypothesis to be invalid.

Question 2. All workers in this field agree that the basic criterion of the sufficiency of a process must depend upon the probability of the survival of a single spore. The F_c value basis of evaluating sterilizing processes uses the probability of survival of a single spore as its criterion. Aside from the indications of experience over many years that this criterion is valid, logic shows the opposing criterion of the number of residual spores per container to be invalid because there cannot be as great a probability of a spore surviving in any food portion away from the critical point as there is of its survival in the food portion which contains the critical point. Calculations which seem to show that there is a greater probability of survival of a spore in food located away from the critical point than in food located at the critical point are based upon a false premise.

Questions 3, 4, and 7. There is extensive experimental and commercial evidence that spores in a suspension with a given concentration are reduced in number to one viable spore by the action of a smaller amount of lethal heat than are similar spores in a suspension with a higher concentration in an identical substrate. The explanation for this fact is not known. A frequently suggested explanation attributes the phenomenon to variability in heat resistance of individual spores. The fact that the rate of destruction of spores is logarithmic, however, requires, under this postulation, the

[f] Calculations of the distribution of lethal heat within a container of a conduction heating product sometimes show the region of least lethal effect to be not the center of the container but an "iso-*j*-region" a short distance from the center. The reason for the existence of this phenomenon is found in the comparatively high lethal effect of the cooling portion of a "still" process of a conduction heating product at the center of the container. Thus, in process calculations based upon conditions at a critical point, heat penetration results for a point a short distance away from the center of the container should sometimes be used. Since this phenomenon is due to cooling lag near the center of the container, it does not appear likely that it will be found in convection heating products.

improbable condition that a fixed relationship must exist between the concentration of spores and the magnitude of the maximum resistance of individual spores. An alternative postulation is that the greatest resistance of spores in higher concentration is due to a mutually protective effect among the spores when they are in close proximity to one another. The answers to questions 3, 4, and 7 all depend upon the explanation of the effect of concentration of spores upon resistance. Until the reason for this phenomenon is known, these questions cannot be answered.

Notwithstanding the fact that answers to some of the seven questions cannot be given, a logical analysis of the problem leads to the conclusion that the probability of survival of a spoilage organism at the point in the mass of food which suffers the least lethal heat effect is the correct criterion of the sufficiency of a sterilizing process.

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ERRATA, VOLUME 20, 1955

Food Research, **20**, 226-237 (1955): The following corrections in this article on thermal process evaluation by Hurwicz and Tischer are noted:

P. 229. The last line erroneously reads: $\frac{U_n}{F_{i_n}} = F_i \pm \frac{U'_n}{F_{i_n}} = F'_n$.

It should read: $\frac{U_n}{F_{i_n}} = F_i \neq \frac{U'_n}{F_{i_n}} = F'_n$.

P. 230. The 4th line (from top) reads: $\sum_1^n \frac{1}{F_i} \pm F'_i \sum_1^n \frac{1}{U'}$

It should read: $\sum_1^n \frac{1}{F_i} \neq F'_i \sum_1^n \frac{1}{U'}$

P. 231. Table 1, last line in Step 2, now reads: log $a_n + 1 =$. . . .
It should read: log $a_{n+1} =$. . . .

P. 233. Fig. 2 of the article as published should be replaced with the new Fig. 2, reproduced below.

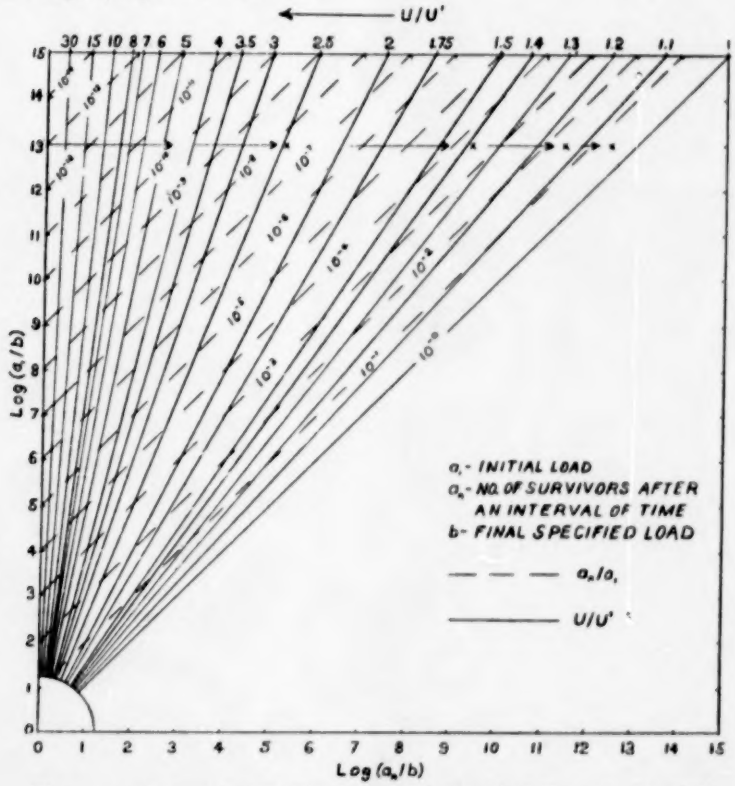
U/U'
30 15 10 8 7 6 5 4 3.5 3 2.5 2 1.75 1.5 1.4 1.3 1.2 1.1 1
Log (a/b)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
a_i - INITIAL LOAD
a_n - NO. OF SURVIVORS AFTER AN INTERVAL OF TIME
b - FINAL SPECIFIED LOAD
a_n/a_i
U/U'
Log (a_n/b)

Figure 2, revised, page 233, Hurwicz and Tischer, Food Research, 20, 1955.

P. 234. In line 5 (from bottom), the parenthesis after . . . calculation) should be moved to the end of line 4 (from bottom).

P. 235. Step 7, last column, reads "Plus Par." This should read: "This Paper."

P. 236. The first line under the table now reads: . . . $= \log a_n - \frac{\triangle t_n}{z_n}$

It should read: . . . $= \log a_n - \frac{\triangle t_n}{Z_n}$

Food Research, 20, 273-281 (1955): P. 274, line 15. This line now reads . . . averaged 12.4° ± 1.4°F. It should read . . . averaged — 12.4°F. ± 1.4°F.

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P. 234. In line 5 (from bottom), the parenthesis after . . . calculation) should be moved to the end of line 4 (from bottom).

P. 235. Step 7, last column, reads "Plus Par." This should read: "This Paper."

P. 236. The first line under the table now reads: . . . $= \log a_n - \frac{\triangle t_n}{z_n}$

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DIFCO

Media for

MYCOLOGY

DIAGNOSTIC

. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:

Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Maltose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

CLASSIFICATION

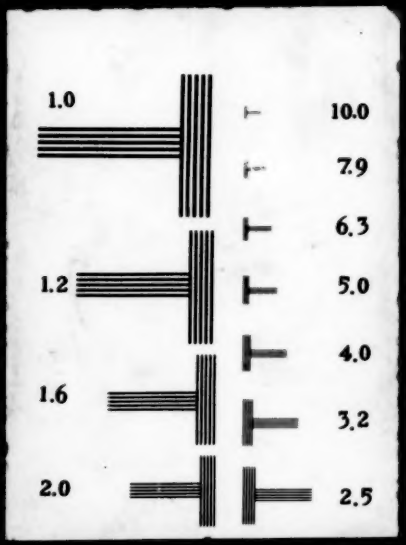
. . . and nutritional studies of fungi:

Bacto-Yeast Morphology Agar
Bacto-Yeast Carbon Base
Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, *NINTH EDITION,*
including descriptions of these media and their use,
is available on request.

DIFCO LABORATORIES
DETROIT 1, MICHIGAN

RESOLUTION CHART



100 MILLIMETERS

INSTRUCTIONS Resolution is expressed in terms of the lines per millimeter recorded by a particular film under specified conditions. Numerals in chart indicate the number of lines per millimeter in adjacent "T-shaped" groupings.

In microfilming, it is necessary to determine the reduction ratio and multiply the number of lines in the chart by this value to find the number of lines recorded by the film. As an aid in determining the reduction ratio, the line above is 100 millimeters in length. Measuring this line in the film image and dividing the length into 100 gives the reduction ratio. Example: the line is 20 mm. long in the film image, and $100/20 = 5$.

Examine "T-shaped" line groupings in the film with microscope, and note the number adjacent to finest lines recorded sharply and distinctly. Multiply this number by the reduction factor to obtain resolving power in lines per millimeter. Example: 7.9 group of lines is clearly recorded while lines in the 10.0 group are not distinctly separated. Reduction ratio is 5, and $7.9 \times 5 = 39.5$ lines per millimeter recorded satisfactorily. $10.0 \times 5 = 50$ lines per millimeter which are not recorded satisfactorily. Under the particular conditions, maximum resolution is between 39.5 and 50 lines per millimeter.

Resolution, as measured on the film, is a test of the entire photographic system, including lens, exposure, processing, and other factors. These rarely utilize maximum resolution of the film. Vibrations during exposure, lack of critical focus, and exposures yielding very dense negatives are to be avoided.